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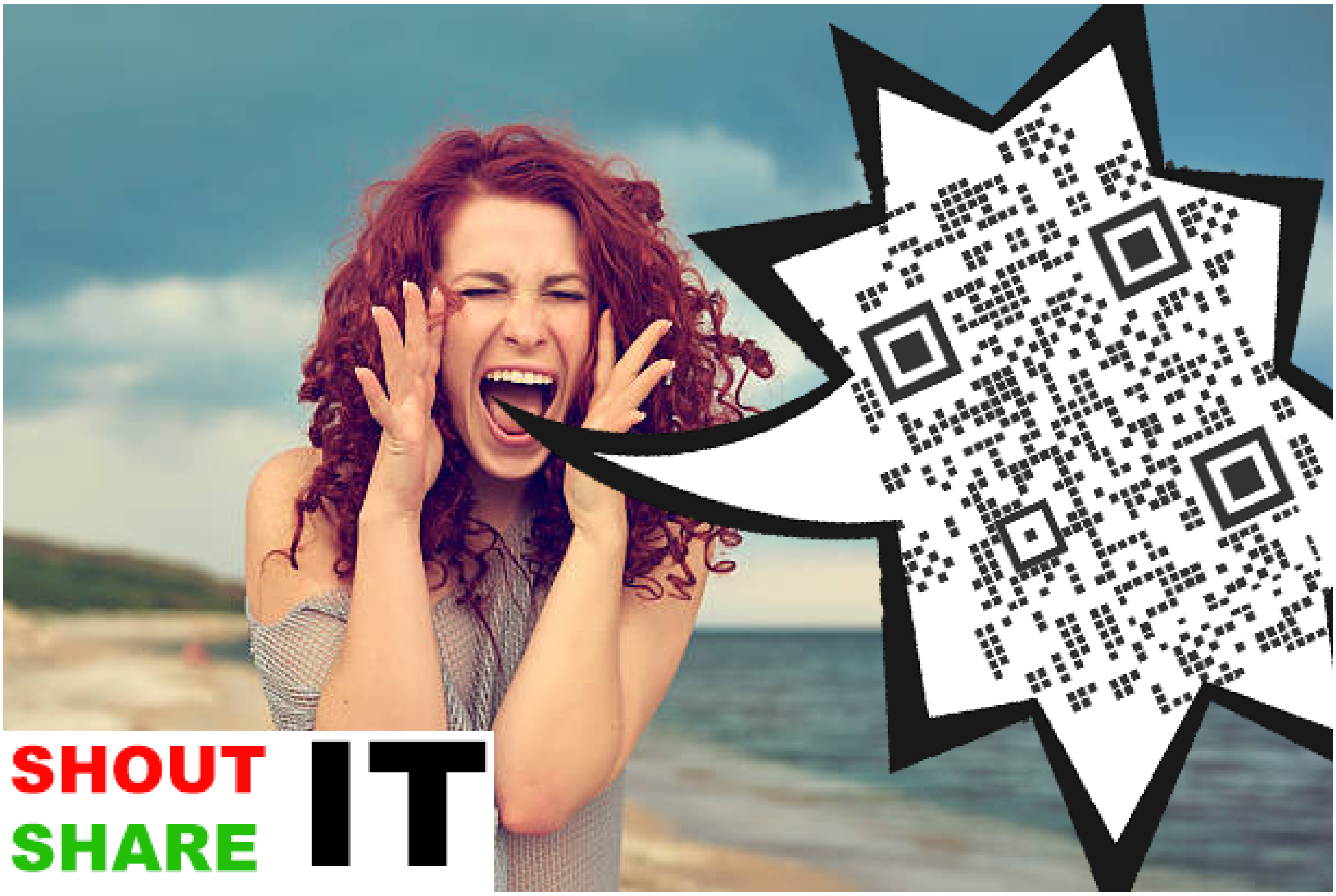
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P. 28, line 1, first column for 17 read 1(?)

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P. 130, line 6 from above, insert after no. 3142.

P. 138, insert as heading: A New Tilletia on Oryza.

P. 180, line 19 from below, for beest read beets.

P. 180, line 9 from below, for 130,000 read 13,000.

P. 201, line 13 from below, for Rugel read Shuttleworth.

P. 251, line 16 from below, for XXI, *fig. 50* read XXII, *fig. 3*.

P. 260, line 11 from below, for — read 436.

P. 261, line 7 from above, for plantlet read plantlets.

P. 261, line 8 from above, after of omit a, and for carpospore read carpospores.

P. 261, line 9 from below, for holdfast read holdfasts.

P. 262, line 2 from above, for *Scytosiphoul omentarius* read *Scytosiphon lomentarius*.

P. 272, line 17 from above, for *Biltmore* read *Raleigh*.

P. 274, line 12, for *hirtefolii* read *hirtifolii*.

P. 352, line 8 from above, for *fig. 40* read *fig. 39*.

P. 353, line 2 from above, for *fig. 41* read *fig. 40*.

P. 353, line 12 from above, for *fig. 42* read *fig. 41*.

P. 353, line 5 from below, for *fig. 43* read *fig. 42*.

P. 354, line 6 from above, for *fig. 44* read *fig 43*.

P. 359, omit line 8 from below.

P. 359, line 7 from below, for *fig. 40* read *fig. 39*.

P. 359, line 6 from below, *fig. 41* read *fig. 40*.

P. 359, line 5 from below, for *fig. 42* read *fig. 41*.

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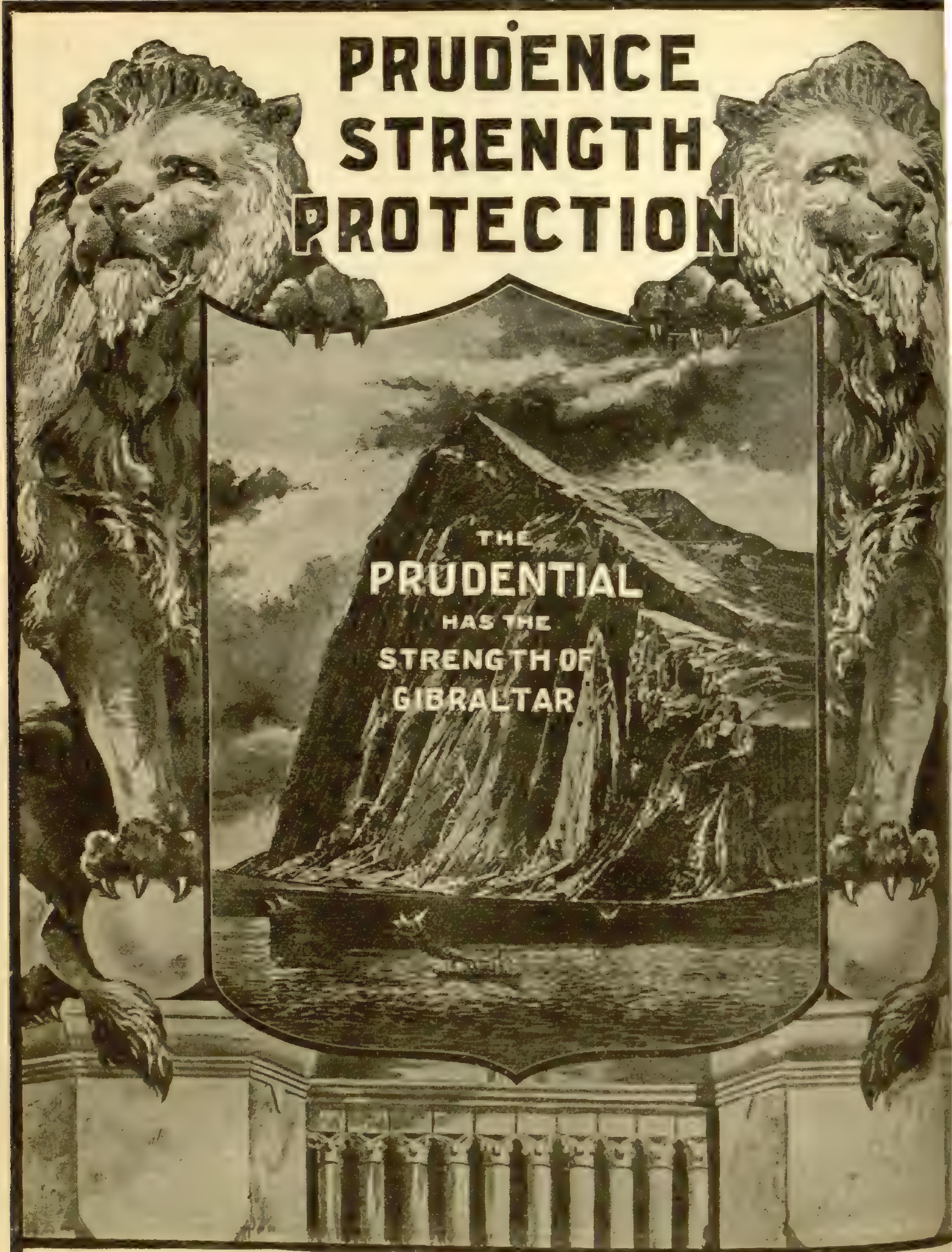
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BOTANICAL GAZETTE

JULY 1899

STUDIES ON REDUCTION IN PLANTS.[1]

GEO. F. ATKINSON.

(WITH PLATES I–VI).

I. REDUCING DIVISION IN *ARISÆMA TRIPHYLLUM* BY RING- AND TETRAD-FORMATION DURING SPOROGENESIS.

In connection with some studies carried on under my direction upon the embryology of certain of the Araceæ, an effort was made to investigate carefully the nuclear figures during the formation of the pollen from the mother cells of the archesporium. In *Arisæma triphyllum* the figures accompanying the heterotypic divisions are of such interest that it seems desirable to offer the results of the study as a contribution to our knowledge of this subject. Especially is this so because the evolution of the heterotypic figures is so divergent from that described for most of the plants heretofore studied; and in one phase coincides so plainly with certain of the types found in animals; while in another phase it departs, so far as I have yet been able to determine, from any type heretofore described.

Early in July the young leaves and the spadix of Arisæma, which are to appear the following spring, are formed, though quite small. They may be found by breaking the aerial shoot from the corm and removing several enveloping fleshy bud scales which protect the next season's leafy shoot. The stamens and pistils appear at this time as very minute protuberances on the surface of the spadix. During the latter part of July and early in August these become more prominent and begin to take on the character of stamens or pistils, as the case may be. The staminate plants can usually be determined during August and in September by the dried remnant of the staminate flower, which in many instances remains attached during the season; or by the cleft in the petiole of the leaf where it emerged. The pistillate plants can usually be determined by the presence of the forming fruit, though some of the smaller pistillate plants of one season become staminate for the next season, because of exhaustion in seed bearing. The archesporium of the staminate plants develops during July, August, and early September; or it may be mature by September.

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[1] Paper read before the Botanical Society of America, at the Boston meeting, August 1898.

The material for this study was collected and prepared by myself during September 1897. The staminate plants were brought to the laboratory and the material was fixed on the day of collection. The spadix was first removed from the bud so that the anthers were entirely exposed. One or two anthers from each spadix were removed and crushed on a glass slip in a drop of water for examination under the microscope to determine the stage of development. In this way all material where the pollen was already developed could be discarded, and only that preserved which was in one or another stage of mitosis. This made it possible to obtain good sections for study on a large number of the slides prepared. The nuclear figures can be quite readily seen in the living condition, but if there was any difficulty in determining the stage of mitosis, a drop of a solution of chloral hydrate was added, as suggested by Humphrey. The spores are formed centrifugally on the spadix, *i. e.*, the stamens at the base of the spadix are usually more advanced than those toward the apex, so that if the pollen was just formed in the lower stamens, the upper ones might show pollen mother cells in one or another of the stages of division. There was, however, considerable variation in the same stamen in this respect, while in a single locule there was little if any variation.

The material selected in this way was placed without further preparation in Flemming's chrom-osmium-acetic solution for twenty-four hours. It was then washed in running water from twelve to twenty-four hours, dehydrated with grades of alcohol; decolorized by placing for twenty-four hours in bulk in hydrogen peroxid and alcohol, made by using 70 parts of a 95 per cent. alcohol and 30 parts of hydrogen peroxid. It was then stored in 75 per cent. alcohol after one rinsing. Six months later a portion of the material was placed in the hands of a student (Miss Susie P. Nichols) for study, and three months later the remainder was prepared on the slide for study by Mrs. W. A. Murrill under my direction. It was imbedded in paraffin, cut with a Minot-Zimmermann microtome 6.6–13.3 μ thick, stained, some in Flemming's triple safranin-gentian-violet-orange stain, and some with iron hæmatoxylin. For the present description of the material, and for the conclusions presented, the writer alone is responsible.

During the formation of the spirem the linin network gradually disappears, and a more or less continuous thread is formed. A distinct spirem was not observed, though this phase of mitosis has not yet been sufficiently studied. Even at the time of the longitudinal cleavage of the thread it was seen to anastomose more or less, where, in its windings, portions came in contact. At this time it presents the appearance of a very open, irregular network. The chromatin granules, at first distributed over the linin network, gradually accumulate in small masses scattered along the thread. As the spirem thread becomes more distinct it shortens, and accompanying this the small masses of chromatin increase in size, giving an appearance of a string of beads, or they present an irregular moniliform appearance. In addition to this beaded appearance of the thread the masses of chromatin are more or less irregular or angular. At some of the angles of the chromatin masses short delicate threads are attached, probably remnants of the linin reticulum which do not participate in the formation of the spirem. This, with the angularities of the chromatin masses, gives a somewhat ragged contour to the spirem. Quite early in the formation of the spirem, and before the reticulum has completely disappeared, the thread shows a longitudinal splitting which is evident between the chromatin masses. In the early stages of the longitudinal splitting of the thread I have not seen the small chromatin masses divided. Each chromatin mass often appears to be made up of two smaller ones lying side by side, which have fused at the points of contact. Even in larger chromatin masses, and among the chromosomes in Arisæma, there is a strong tendency for those lying in contact to fuse more or less, so that the individuals of a single mass or of the chromosomes are often more or less obscured.

While the chromatin masses on the spirem are more or less fused, they are small and emarginate at opposite ends, and at a point coincident with the axis of the band. By this character one can distinguish the double nature, or paired condition of the masses. It suggests a division of the original masses, or the deposition of the chromatin in paired masses along the thread, which fuse more or less at the point of contact. These chromatin masses often stain unevenly, two portions separated by the axis of the thread or band taking on a deeper stain. The darker points lie in pairs along the thread, each separated by a paler chromatin area which runs for a short distance on either side along the division of the thread. At the point between the deeper staining masses the paler zones fuse.

As the spirem shortens, these paired chromatin masses are brought nearer together. Fusion among them takes place, so that the masses increase in length and breadth as the spirem broadens. The longitudinal division of the spirem is marked here and there, sometimes at quite regular intervals, by rounded, elliptical, or oblong openings in the chromatin, while in other places, for considerable distances, no line of division can be ascertained, though often the difference in the intensity of the stain marks the separation of the masses into pairs, while the paler surrounding zones are fused. As the band shortens, the contour becomes more irregular, showing crenations and irregularities, to which are often attached delicate strands, perhaps portions of the linin reticulum.

The chromosomes are marked off by the band separating into sixteen segments. So far as could be determined, this number is quite constant. The segments are not formed by an abrupt division of the spirem, but it appears as if the chromatin masses by shortening unite themselves into elongated areas, which are connected for a time by two delicate threads representing the original line of longitudinal division. The chromosomes lie around the periphery of the nuclear cavity and are quite variable in form; this is due to variations in the extent to which longitudinal fission has proceeded. In a few, longitudinal fission is complete, and a pair of long knotted rods is present. In others, there is a slight connection at one end or near the middle by the fusion of adjacent chromatin. In others, one or both ends of the flattened chromosome are indented or forked; in the latter case figures approaching the letters Y or X are formed. In many of the chromosomes the line of fission is shown by one or two small rounded openings or narrow open slits near the middle or near the ends; or the opening is long and extends from end to end, individuals of the pair being connected only at the end, thus resembling long irregular chain links. Some of the individuals of the pair lie parallel; others are separated more or less at the middle, when quite well defined rifts are formed. Still others resemble chain links twisted half way around, thus presenting a figure 8 appearance (*fig. 6*).

The length of the chromosomes is now from one third to one half or more the diameter of the nuclear cavity, and they show the irregularities presented by the spirem band, being knotted or angular along the edges or at the end. Delicate linin threads are often still attached at the knotted or angular enlargements.

The chromosomes now shorten, the chromatin becoming massed together, until their length is from one sixth to one fourth the diameter of the nuclear cavity. A larger proportion of them have now opened out in the middle in the form of rings, and, while they have shortened in length, their breadth is about the same as formerly, or they may be a little broader. This gives a more perfect ring form, but the chromosome is still somewhat longer than its breadth, so that the line of longitudinal fission can be determined. The line of longitudinal fission is also indicated in other ways. Other chromosomes at this time are present in the form of short plates, some of which show slight indentations at the end, while in others longitudinal fission is complete, and a pair of rods is formed.

During the process of shortening of the chromosomes, the greater part of the chromatin usually becomes massed at four definite points in the ring, or plates, or pair of rods. These denser masses of chromatin lie near the ends of the rods, or near the ends of each lengthened half of the ring or plate, and are connected by paler staining areas, the density of which varies in different rings. These four masses of chromatin, in the ring or in the rods as the case may be, form the tetrad. These rings and tetrads in *Arisæma triphyllum* are very distinct, as much so as in certain animals. The two lengthened halves of the rings are usually more nearly separated along the line of longitudinal fission than along the line of transverse division, so that they quite readily separate. Each half of the ring is more or less crescent shaped, and the ends, instead of being rounded, are often quite regularly angular, presenting figures that frequently aid one in determining the orientation of the cleavage line. Up to this time the chromosomes still occupy their position around the periphery of the nuclear cavity, and, up to quite a late period, often show fragments of linin attached.

The nuclear membrane now disappears, and the kinoplasmic threads, which are to form the spindle, enter and move the chromosomes up to the nuclear plate. The threads first radiate irregularly in all directions, but converge more and more at two poles as they gradually form the spindle. As this takes place, there seem to be two centers of force which lie at the poles of the spindle, and occasionally this is manifested a short time before the spindle is complete by radiations of kinoplasm about the poles in the form of rays, which suggests a centrosphere figure (*fig. 14*). This was observed very plainly in one case, and perhaps similar figures in other plants have led observers to interpret them as centrospheres.

As the chromosomes are drawn to the nuclear plate they lie in various positions. At first it was thought that the axis of the chromosome, whether a ring, a pair of rods, or plate, lay perpendicular to the axis of the spindle. I have observed them in this position a short time before the spindle is complete. In some such cases, the position of the spindle threads suggests that the chromosomes are pulled around before division so that the axis is parallel with the spindle axis. In other preparations, the chromosomes, where they lie close together, tend to fuse to such an extent that it is impossible to determine in what position they lie at the nuclear plate, *i. e.*, whether parallel with or perpendicular to the axis. In a large number of preparations conditions are such as to lead me to believe that the axis of the chromosome is parallel with the axis of the spindle. The chromosomes are so numerous and of such size that they cannot all lie at the periphery of the plate, but occupy the center of the nuclear plate as well, so that an end view of the nuclear plate shows the chromosomes quite evenly distributed over this area. In many cases a large number of them are fused, and curious figures are thus presented. At other times they lie entirely separated, so that the individual rings, or pairs of rods, can be seen and counted in an end view of the nuclear plate. Where the section is cut so as to show an oblique view of the plate, the groups of tetrads, or pairs of rods, can be well seen and counted. From studies of sections in this direction, it appears that there are sixteen groups of tetrads, which would make sixty-four individual chromosomes, thirty-two to be distributed to each daughter nucleus of the first division, and perhaps sixteen to each daughter nucleus of the second division. A side view of the spindles of such preparations, at the nuclear plate stage, shows the long axis of the rings or pairs of rods to be parallel with the axis of the spindle. Since the chromosomes are distributed through the center of the nuclear plate as well, they lie like a bundle of chain links. The form of the ring, as suggested above, indicates that the axis of the longitudinal cleavage of the spirem or of the tetrad lies parallel with the axis of the spindle.

This is important in determining the character of the first division, as to whether it is to be a longitudinal or a transverse division of the chromosomes.

Not only does the form of the ring aid in determining the position of the chromosomes at the nuclear plate stage of the first division; the paired rods also serve to determine this. These lie parallel with the axis of the spindle. Even before the rings have moved to the nuclear plate there is a great tendency for them to separate into longitudinal halves. This occurs also at the nuclear plate stage. The tetrads separate more readily along the line of longitudinal division than they do on the line of transverse division. For this reason, at the nuclear plate stage, the figures with paired rods, rings partly or completely separated along the line of the lengthwise cleavage of the spirem, together with the form of the rings, should determine the position of the long axis of the chromosomes. The tufts of spindle fibers are attached to each individual of the tetrads, and transverse or reducing division of the chromosome results as these are drawn away from the nuclear plate. In many cases the sixty-four tetrads, thirty-two to each pole, move as distinct individuals, so that longitudinal, as well as transverse division, occurs in the first mitosis of the mother cell nucleus. But while transverse division occurs during the first mitosis of the nucleus, longitudinal division of the chromosome takes place first. The peculiarity of Arisæma, then, is that, while longitudinal division precedes transverse division of the chromosome, both divisions occur during the first or heterotypic division, and the real reduction follows soon the pseudo-reduction in the heterotypic division.

The only other account of a reducing division in the first mitosis which I have noticed is that by Korschelt in the study of the development of the egg of the annelid Orphryotrocha, quoted by Wilson in "The cell in development and inheritance," p. 201. During the first mitosis in oogenesis, the spirem segments into four chromosomes, which is the normal number in the somatic cells. These split, and then the pairs fuse together again, the four chromosomes arranging themselves in a tetrad, two going to the first polar body and two remaining in the egg cell. These two split and form another tetrad, two chromosomes going to the second polar body and two remaining in the egg nucleus. This brings about reduction in the first mitosis.

Beliaieff[2] for a number of years has maintained that a reducing division takes place in plants, and, based on his studies of the division of the pollen mother cells of Iris, classifies the modes of division of the nucleus in plants into three types: (1) the vegetative division; (2) the heterotypic division; and (3) the reducing division. The heterotypic division takes place, as is well known, in the first mitosis of the pollen mother cell, and the reducing division accompanies the second mitosis.

Calkins[3] announces reducing division during the second mitosis of the pollen mother cell in Pteris and Adiantum. Calkins was unable to determine whether the reducing division in Pteris and Adiantum occurred during the primary or secondary mitosis, since the tetrads were packed so closely in the nuclear plate of the first division. During the secondary mitosis the diads elongate to form a rod. This elongation he thinks takes place in the direction of the axis of the spirem, and would thus show that the primary mitosis is an equation division, while reduction takes place during the secondary mitosis. Nevertheless he recognizes that it is immaterial whether reduction takes place during the first or second division. The formation of tetrads in the prophase of the primary mitosis is generally regarded as the preparation of the number of the chromosomes which are to be eventually distributed to the four daughter nuclei of the second mitosis; and where the tetrads are formed by one longitudinal and one transverse division of the segment of the spirem, it is equivalent to reduction in the prophase of the first division, though usually, so far as accounts go, the final separation for the reduction does not occur until the second division. In Arisæma the mode of tetrad formation by longitudinal and transverse division is such as to effect reduction in the prophase of the first division, while during metaphase and anaphase the reduction is completed by the separation of the tetrad of the transversely divided diad.

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[2] BELIAIEFF, W.: Ueber die Reductionstheilung des Pflanzenkernes (Vorläufige Mittheilung). Ber. d. deutsch. bot. Gesells. **16**: 27–34. 1898.

[3] Chromatin reduction and tetrad formation in pteridophytes. Contrib. Dept. Bot. Columbia Univ. **5**: 101–115. *pl. 295, 296.* 1897.

II. REDUCING DIVISION OF THE CHROMOSOMES IN *TRILLIUM GRANDIFLORUM* DURING SPOROGENESIS.

In studying the development of the pollen of *Trillium grandiflorum*, the chromosomes were found to be of such large size as to offer, possibly, a good opportunity for an investigation which might throw some light on certain of the problems of mitosis during sporogenesis. The preliminary studies were made upon material collected during the autumn and winter of 1896–7. With the experience of one season's work it was possible to plan for the collection of a quantity of material in different stages of development. The young flowers of Trillium begin their development in June and July of the previous year, and by autumn they are well formed, though they remain protected in the sheathing bud scales during the winter. So far as I have found, the archesporium is completed and the period of growth of the pollen mother cells is closed with the oncoming of winter, if not before. The period during which the division of the pollen mother cells takes place extends over seven or eight months. This is due not only to some variation in the time of maturing of different plants in different localities, but also to temperature. In the open woods, on rather high ground, where the plants are protected from cold north and west winds and are exposed to the warmth of the sun, the pollen is often mature in the latter part of September. In the cool ravines and open places exposed to north winds, the pollen is formed in warm days during February, March, or April, according to the season, and to some extent also according to the state of maturation in different individuals.

Very cold weather checks the progress of mitosis, but when the mother cells have reached the proper state of maturation a few warm days in the winter or early spring months are sufficient for the two successive divisions. It appears that during the winter season, when the nights are cool and the days warm, the mitotic figures may be prolonged for several days. For this reason it is not difficult to prepare a large quantity of material in different stages of division. For the present study the material was collected during the month of February 1898. Some of this was growing in a garden where it was transplanted the year before, while the bulk of the material was collected from wooded ravines, from one to two miles distant from the laboratory, the plants being easily found by raking off the leaf covering on the ground.

All the material was examined before fixing in order to know just the stage of division in individual plants, and even assorted into lots showing the spirem stage, first and second division, for convenience in cutting material of any desired stage. During division the chromosomes are so large and the cytoplasm so clear, one can easily determine the different stages with a one sixth objective, so that desired material could be assorted into that showing prophase, nuclear plate, anaphase, etc. In each individual flower, the tip of one of the stamens was removed, crushed in a drop of water on the slide, and examined first with a two thirds objective, then with a one sixth. In this way undesirable material could be rejected.

Before placing in the Flemming solution, sometimes the tips of all the anthers were cut off, while other material was placed in the fixing solution with the anthers closed. After the removal of the sepals, petals, and the apex of the pistil, so that the fixing solution could enter the locules of the young carpels, the entire andrœcium still attached to the receptacle was thrown into the solution. This made it possible to section several anthers of an individual flower together.

The fixing solution used was Flemming's chrom-osmium-acetic solution. The material remained in this from fifteen to twenty-four hours, was washed from twelve to twenty hours in cold running water, dehydrated in grades of alcohol to 95 per cent., then decolorized in mass in alcohol of 70 per cent. which was diluted with hydrogen peroxid. It was then passed back to alcohol of commercial strength, then through absolute alcohol, and cedar oil ; finally it was infiltrated with paraffin and imbedded. It was then stored in the paraffin blocks for a few weeks or months as time became available for cutting and staining. The material was cut on a Minot-Zimmermann microtome and stained, some with the triple stain (safranin-gentian-violet-orange) and some with iron-hæmatoxylin.

The early stages of the spirem have not yet been studied, but the spirem forms a broad and probably continuous band. Before it segments, it often shows a differentiation, where not overstained, into a ground substance giving a pale purple or pale violet reaction with the gentian violet, and more deeply stained bodies which are in pairs and appear at quite regular intervals in the band. The line of separation which runs along between the pairs of denser chromatin masses marks the line of longitudinal division of the spirem, though the ground substance often shows no division line. As the spirem matures, here and there are seen short openings along the middle line. This is especially well marked as the spirem segments into the chromosomes. The individual chromosomes often show traces of longitudinal division, by short openings near one end, or at the middle, oftener at the ends, where there is a slight indentation ; or, the division proceeding deeper, the end is more or less forked, resulting in Y- or U-shaped figures.

The spirem segments into about six chromosomes. They are at first rather long and become somewhat shorter and broader as they move to the nuclear plate of the spindle.

At this time the usual changes in the nucleus take place. The membrane disappears and the threads of kinoplasm move in, showing first a radiating arrangement and gradually moving to converge into the two poles as the spindle is formed ; the chromosomes are drawn toward the center and are finally oriented in the nuclear plate. The chromosomes are broad, flattened, and irregularly oblong. There is considerable variation in size. They are very characteristic in form and structure. A few show narrow slit-like openings in the middle. A few of this form become very short and the opening then is somewhat rounded, so that a ring form is the result. This is comparatively rare. Others are divided at the ends, some showing a slight emargination, while some are more or less deeply forked, showing a tendency to form X and Y figures. Combinations of these two types are also found, so that there will be an opening in the middle, while the ends are somewhat forked, or one end is deeply divided. More characteristic, however, is the tendency to a differentiation in the density of the chromatin, which is especially marked when the chromatin is not stained too deeply. This differentiation of the chromatin is of the same kind as that manifested in the spirem and shows the paired condition of the chromosomes. The ground substance of the chromosome in these cases shows uniformly a paler tint and is translucent, while the denser masses of chromatin stain very dark and are more or less opaque. These chromatin masses are paired just as they are in the spirem, and probably result from the division of single chromatin masses in the early fission of the spirem. When these chromatin masses are well marked in the chromosomes, they seem to be uniformly of the same number so far as observed. There are often found well marked pairs of chromatin masses, each mass lying in the edge of the bar, four at the ends (two at each end) and four near the middle (two on each side near the middle), making eight in all.

Very frequently the edge of the bar is undulate, the prominences on the edges occurring at the location of the chromatin masses. The apparent uniformity in the number of these chromatin masses in the chromosomes is, perhaps, of considerable interest, and one is led to inquire whether they represent the units of the chromosomes or whether each one is a member of a tetrad group. If the latter is the true interpretation, then there would be in each chromosome of *Trillium grandiflorum* two united tetrads, or the chromosomes in the prophase of the heterotypic division would be quadrivalent, instead of bivalent, as in the case of the normal tetrad.

As the chromosomes are drawn toward the nuclear plate, they become bent, so that each one represents a short arc of a circle or a broad open U form, as if drawn more forcibly by threads attached to the middle portion. As they are oriented on the nuclear plate transverse to the axis of the spindle they at first stand in various positions. Some lie tangentially, with the convex side toward the axis of the spindle and with both ends at the periphery, while others lie so that one end is directed toward the axis, with the other end at the periphery. In this way they are sometimes "convolute" or more or less "imbricate" in the nuclear plate, and may remain so for some time during the metaphase, or while separation at the nuclear plate is beginning to take place. Before separation has proceeded far, however, the chromosomes are usually more bent upon themselves, with the free ends of the U nearer together and directed outward in the usual way.

While the monaster is forming, and many of the chromosomes stand with one end directed toward the axis of the spindle and the other radiating therefrom, certain ones which are strongly lobed at the ends present a figure which leads one to think that the ends might be separated first, and that the chromosomes might then, in the anaphase, move to the poles in the form of a U, but with the concave or open side directed toward the poles, or that the inner ends might be separated first, and the chromosomes then move to the poles in the rod form. It does not seem, however, that the movement of the chromosomes has begun in such figures, and it is suggested that they are not yet drawn into proper position in the nuclear plate.

As separation of the paired chromosomes begins, the larger number of spindle threads appear to be attached in one tuft at the middle. The flattened U-shaped chromosome now broadens at the middle, in response to the tension of the tuft of threads attached at this point on either half, and the chromatin substance is soon drawn out into a short process on either side which becomes the point of the V-shaped daughter chromosome, or forms a slight projection on the convex side of the U-shaped ones if this form is retained. No pull is exerted on the end of the chromosome, the ends of the pairs remain united, or in position at the periphery of the nuclear plate, while the middle portions are drawn toward the opposite poles, and in opening out thus, the dividing chromosome forms the diamond-shaped figure when seen from in front (*fig. 18*).

During the anaphase the chromosomes are V-shaped or U-shaped, and show considerable irregularity in contour, being more or less nodulose, often showing still the four dense chromatin masses. Some close up behind and form rings, some divide in front and form two rods. As the chromosomes approach the poles the polar ends converge, so that the chromosomes lie close together around the periphery of the ends of the spindle. Lying in this position the daughter nucleus is formed. The form of the daughter nucleus is somewhat like the half of a biconvex lens, the convexity being outward, while the truncated end lies toward the cell plate now formed by the connecting spindle threads. The form and position of the chromosomes give to the daughter nucleus its shape, for as the closed ends of the V- or U-shaped chromosomes converge at the apex of the cone, the spreading arms of the open end cause the nucleus to broaden out on the side facing the cell plate. The nuclear cavity now appears and the chromosomes are lying on its periphery against the nuclear membrane. They become usually more irregular in form, with angular points on the edge to which appear to be attached delicate threads connecting with the nuclear membrane, or reaching to an adjacent chromosome.

Where the nucleus is small by the close crowding of the large chromosomes, it is quite impossible to determine whether the chromosomes unite in such a way as to form a spirem. The nucleus does not, however, pass into a resting stage with the linin reticulum upon which the chromatin is distributed, but the chromatin bands remain intact. In the large nuclei, where the chromosomes do not lie so close together, they appear in most cases to remain distinct, and in the form of V- or U-shaped bands, or often they are horseshoe-shaped, and sometimes form rings. In some cases it appears as if the arms of adjacent chromosomes coming in contact had fused at the end, but in no case could I see that they were thus united around the nucleus to form a continuous spirem, as described by Mottier for Podophyllum. Sometimes it appeared as if two had united by their ends to form a very large ring. In other cases the chromosomes may be permanently separated into two groups during this period.

As the nuclear cavity is formed and the first spindle is disappearing, the free ends of the chromosomes sometimes bend inward partly over the truncate side of the cavity, and at other times the chromosomes do not occupy the regular position which they usually show when they have reached the poles. In these cases ends of the chromosomes may be fused here and there, but in all stages several free ends are to be seen, and the figures presented by the nucleus are such as to lead one to believe that the chromosomes remain distinct through this phase. If the daughter nucleus is not elongated when first formed, it very soon begins to elongate in a plane parallel with the cell plates, so that it becomes nearly or quite twice as long as its diameter, and it is more or less inequilateral, the convex side being toward the periphery of the primary mother cell, while the plane side faces the cell plate. In opening out in this way the chromosomes become more and more distinct. The elongation of the daughter nucleus often takes place while the chromosomes are moving to the poles (*fig. 21*). In such cases the chromosomes are more easily followed, and the evidence is quite convincing in support of the view that the individuality of the chromosomes is preserved from the anaphase of the first division through to the prophase of the second mitosis. From the V- and U-shaped forms possessed by the chromosomes as they go to the poles, many of them change to horseshoe form, or some to complete rings by the free ends converging while the arms part slightly at the middle portion. In a number of cases the chromosomes divide transversely at the convex end and the two rods lie side by side or near each other. The nuclear membrane now disappears and the kinoplasmic threads enter to form the spindle for the second division.

The elongation of the nucleus while the chromosomes are moving to the poles shows that the forces are in play which form the spindle for the second division. It is evident also that the chromosomes remain distinct, and that they are soon to be again separated transversely.

The elongated form of the nucleus marks the position of the spindle for the second division, and the poles of the spindle lie near the poles of the elongated nucleus. The spindle is therefore inequilateral, the poles being curved toward the cell plate of the first spindle figure. At the same time the chromosomes begin to move inward to the cell plate, and in doing so show the same general form which they possessed during the anaphase of the heterotypic division, which it seems they possess through the short period which intervenes before the formation of the second spindle figure.

During this time there is no longitudinal cleavage of the chromosomes. Even should the chromosomes form a continuous band in the daughter nucleus of the first division, there is no longitudinal splitting of the same at this time. Sometimes there is an appearance of a longitudinal fission of the chromosomes where the edges seem to be more deeply stained than a middle zone along the axis. This was found to be due, however, to the deeper staining of an outer layer of the large chromosome, and in properly stained preparations can be seen at any stage of mitosis.

If longitudinal fission of the chromosomes takes place during the second division, it would then be sought for in the nuclear plate stage.

Since the chromosomes during the prophase of the second mitosis are of the same general form as those of the anaphase of the first division, and very likely preserve their identity through the short intervening period, they should be chiefly V- or U-shaped. This is the case, as the examination of a large number of preparations proves, while a few are ring form (*fig. 30*), and still others, having divided transversely at the apex of the V or the closed end of the U, exist as a pair of rods.

As the chromosomes approach the nuclear plate the arms of the V- or U-shaped ones close together, and, usually at the same time, transverse division takes place at the closed end. In this way all the chromosomes become of nearly uniform shape, consisting of two parallel or nearly parallel rods, which become more or less fused along the line of contact. The result of this is to form a double chromosome, very broad and irregularly oblong, which resembles in a striking manner the paired chromosomes at the nuclear plate of the first or heterotypic division, though they are formed as a result of folding and transverse division instead of by longitudinal cleavage. As the paired chromosomes are oriented on the nuclear plate, they usually become bent in such a way that the convex side lies toward the axis of the spindle, while the ends are directed outward, and the axis of the double chromosome is perpendicular to the axis of the spindle. The figure therefore presented by the metaphase of the second division is very much like that of the heterotypic division, and the only way in which one can determine that these belong to the second division is by the fact that there are two such spindles within the wall of the mother cell. Variations in their position in the monaster occur. One end of the chromosome may be directed inward toward the axis of the spindle, so that a few of them may be somewhat convolute; or the bent end may lie tangentially at the periphery of the spindle; or the chromosome may be nearly straight and standing on one end, while the other end radiates outward. As the position of the chromosome varies at the nuclear plate, so the figures presented during the anaphase vary, but the result is the same in each case.

The broad chromosomes lying thus at the nuclear plate, their edges face the poles of the spindle; the threads of the spindle which pull on the chromosomes are attached to that portion of the chromosome on either side which lies near the periphery of the spindle. On those which are bent so that both arms of the

U radiate equally, the threads are attached at the middle; on those which stand so that the arms radiate unequally the threads are attached somewhere between the end and the middle; while those which stand on the end have the threads attached at the end of each edge. The pull of the threads attached at the different points on the differently oriented pairs of chromosomes separate the individuals of the pair, the separation beginning at the point of attachment of the thread, and, as the portions are drawn toward the poles, the liberation proceeds until the members of the pair are no longer in contact. The different figures presented may be grouped all in three types, the V or U, the hook or the V with unequal arms, and the straight rods. These as can be readily seen are dependent on the point of attachment of the spindle threads.

The result of this separation of the individuals of the paired chromosomes in the second mitosis is a reducing division of the chromosomes, or a qualitative reduction of the chromatin substance; for, as we have seen, the paired chromosomes in the second mitosis are formed by the looping of longer chromosomes, which often open out at the bent end before reaching the nuclear plate. The second division results simply in the separation of the arms of this loop, and the distribution of each to a different daughter nucleus. It may be admitted here, then, with a feeling of reasonable confidence, that in *Trillium grandiflorum* a reducing division of the chromosomes, or in other words, a qualitative reduction of the chromatin takes place during the second division of the nucleus in the development of the pollen or microspores. One may well be cautioned against a hasty judgment in the interpretation of the figures presented during sporogenesis, because of what seems to be contradictory evidence given by different investigators upon the question as to whether or not a reducing division of the chromosomes takes place in plants, and it is only after careful study of an excellent series of preparations that I am led to present this as my conviction of the nature of the process as it occurs in Trillium. The large size and small number of the chromosomes, as well as their form, have contributed in no small degree to the results obtained.

As is well known, the investigations of Mottier[4] upon division of the pollen mother cells in Podophyllum, led Strasburger[5] to believe that a reducing division of the chromosomes occurred during the second mitosis of the pollen mother cell. But more recent researches by Mottier, especially upon the condition of the chromosomes in the daughter nucleus and the origin of the chromosomes for the second division, led Strasburger[6] to recede from his former position and to reaffirm his conviction that the second division is the result of a longitudinal cleavage. According to Mottier, the chromosomes unite in the daughter nucleus of the first division in such a way as to form a continuous single band, *i. e.*, a band made of single chromosomes united end to end, the arms of the V-shaped chromosomes and of the paired ones, where transverse division has taken place, open out to join with the arms of neighboring ones. This band forms the spirem, which splits longitudinally before it segments into the chromosomes for the second division. As a result of this process of longitudinal fission of the spirem in Podophyllum, the segments or chromosomes are paired, but the pairs in the case of Podophyllum would then be the result of longitudinal fission, The separation of the members of the pair at the nuclear plate during the second mitosis would not then be here a reducing division.

These conclusions of Strasburger and Mottier led me to study very carefully the same stage in Trillium. As I have indicated above, the evidence seems to me to show that the chromosomes retain their individuality through the daughter nuclei from the anaphase of the first division to the prophase of the second. It might, however, be admitted that they unite to form a continuous spirem, without invalidating the conclusions reached in this study, for I have not found the slightest evidence of a longitudinal fission during the daughter nucleus stage. On the other hand the chromosomes, as the nucleus opens out, appear in the same form and number as shown at the close of the first division, that is, in the form of a letter V or U, or in rings, or even in the form of paired rods, which form more or less the same figure, and agree in this respect with the same types described by Beliaieff for the same stages.

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[4] MOTTIER, D. M.—Beiträge zur Kenntniss der Kerntheilung in den Pollen-mutterzellen einiger Dikotylen und Monokotylen. Jahrb. f. wiss. Bot. **30**: 169–204. *pl. 3–5.* 1897.

[5] STRASBURGER, E.—Ueber Cytoplasmastructuren, Kern- und Zelltheilung. Jahrb. f. wiss. Bot. **30**: 375–405. 1897.

[6] STRASBURGER, E. and MOTTIER, D. M.—Ueber den zweiten Theilungsschritt im Pollenmutterzellen. Ber. d. deutsch. bot. Gesells. **15**: 327–332. *pl. 15.* 1897.

The origin and form of the chromosomes in the first division of the pollen mother cell of Trillium, and the figures presented at the nuclear plate, show that the first division is heterotypic, though the transverse division of the chromosomes at this time, which indicates the tetrad character, is rarely present. The figures presented by the second division are exceedingly interesting, since they suggest the heterotypic division also, as is indicated by rings, in some cases, which are formed by the closing of the open ends of a V or U figure. There is thus a semblance of a heterotypic figure, with reducing or transverse division during the second mitosis in the sporogenesis of Trillium, and it would be interesting to know if the heterotypic figure described by Farmer[7] during the second mitosis in the sporogenesis of some liverworts, results in qualitative reduction. The figures presented by the chromosomes at the nuclear plate of the second division are strikingly similar to those which exist during the true heterotypic mitosis, and the separation of the chromosomes gives, during the anaphase, figures exactly like those shown during the first division.

A larger number of chromosomes in the second division, perhaps, are of the hooked form, and some are of the rod form, though both the hook and rod form are found rarely in the first division. It would appear that in Trillium, as well as in Arisæma, as shown by my studies on reducing division in that genus, the form of the chromosomes and the mode of separation at the nuclear plate would not permit of classification into the types suggested by Beliaieff.[8] It is quite reasonable to believe that in mitosis, where the size and proportional dimensions of the chromosomes vary, as they are so well known to do, a great variety in the form of the chromosomes may exist, and that, correspondingly, there may be great variation in different species manifested in chromatin figures and evolutions, even where the same result is finally obtained.

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[7] Farmer, J. B.—On spore formation and nuclear division in the Hepaticæ. Ann. Bot. 9:469–523. *pl. 16–18.* 1895.

In the peculiarities of the second mitosis of the pollen mother cell in *Trillium grandiflorum*, there is presented a distinct type in the evolutions of the chromosomes during the reducing division. Considerable interest attaches also to the peculiarity of the large chromosomes during the anaphase and metaphase of the first mitosis, where there is an appearance of eight denser portions of chromatin in the chromosomes, arranged in four pairs in such a way as to suggest two tetrad groups in a single segment of the spirem. In connection with this, it is interesting to note, that, while in *Trillium grandiflorum* during the first mitosis in the pollen mother cell there are only six chromosomes, in a number of the Liliaceæ there are twelve. This indicates, possibly, that the segments of the spirem in Trillium here represent four chromosomes, fused end to end (quadrivalent), instead of two (bivalent), which is usually the case as a result of the pseudo-reduction. *Arisæma triphyllum*, according to my studies on this species, also presents a distinct type, in that the reducing division, though following the longitudinal division, occurs during the first mitosis.

That there should be variations in the evolutions performed by the chromosomes in different plants, such as to represent different types, is what one might expect, not only in view of the great variations in the size and form of the chromosomes in different plants, representing different types of chromosomes, but also in view of the tendency to variation so manifest in many of the phenomena of plant life, extending even to nuclear phenomena, illustrated by the different types in fertilization, as shown by the investigations of Ikeno[9] in Cycas, Shaw[10] in Onoclea, and Blackman[11] in *Pinus sylvestris*, as well as by Miss Ferguson[12] in *Pinus Strobus*.

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[8] BELIAIEFF, W.—Ueber die Reductionstheilung des Pflanzenkernes (Vorläufige Mittheilung). Ber. d. deutsch. bot. Gesells. **16**: 27–34. 1898.

Is it not well to inquire if some of the divergent and contradictory results regarding the behavior of the chromosomes obtained by different investigators, when dealing with different plants, are not due to the fact that we are dealing with different types in some cases? Are there not among plants different types of chromatin reduction? So that in one type there is represented a mass reduction, or quantitative reduction of the chromatin; in another type a pseudo-reduction, or numerical reduction only of the chromosomes; and in another type a qualitative reduction of the chromatin or reducing division of the chromosomes? Touching the hereditary or constitutional influences of fertilization, we recognize different types in plants, as shown by close- and cross-fertilization; and different types also in the mechanism for bringing about pollination.

Some of the bewilderment which now surrounds certain phases of the study of the morphology of the nucleus will, I believe, disappear, if we recognize that there is such a thing as a reducing division or qualitative reduction in plants as represented by such types as Trillium, Arisæma, Adiantum, Pteris, Iris, etc.; that there are plants in which only a quantitative or numerical reduction occurs, represented by such a type as Podophyllum; and possibly that there is still another type, where in the same plant qualitative reduction may take place in some cells, while quantitative or numerical reduction only takes place in others. This seems to me, as a working hypothesis, more reasonable than to insist, because one type has been found in one or several plants, that all plants must conform to it.

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[9] IKENO, S.—Untersuchungen über die Entwickelung der Geschlechtsorgane und den Vorgang der Befruchtung bei *Cycas revoluta*. Jahrb. f. wiss. Bot. **32**: 557–602. *pl. 8–10*. 1898.

[10] SHAW, W. R.—The Fertilization of Onoclea. Ann. Bot. **12**: 261–285. *pl. 19*. 1898.

[11] BLACKMAN, V. H.—On the cytological features of fertilization and related phenomena in *Pinus sylvestris* L. Phil. Trans. Roy. Soc. B. **190**: 395–426. *pl. 12–14*. 1898.

[12] Miss Ferguson's studies were carried on under my direction in the Bot. Lab. Cornell University, and a paper, yet unpublished, was read before the Bot. Soc. Am. Aug. 1898, entitled "A preliminary note on fertilization in the white pine."

CORNELL UNIVERSITY.

EXPLANATION OF PLATES I–VI.

(All the figures are drawn to the same scale, using a Zeiss microscope, compensation ocular 12, and the 2^{mm} homogeneous immersion objective, the image being thrown down even with the base of the microscope. The figures are reproduced from these drawings without any reduction.)

PLATES I AND II. Arisæma triphyllum.

FIG. 1. Spirem stage of nucleus showing the dividing thread with chromatin masses on it. The masses of chromatin are partly divided, and appear as paired masses along the thread.

FIG. 2. Portions of partly formed spirem, showing indications of longitudinal division.

FIG. 3. Early stage of chromosomes, just after segmentation of spirem band, showing different stages of longitudinal division, forming oblong plates with ends slightly forked, or those approaching X and U forms, where the division is more marked. Fragments of linin are still attached.

FIGS. 4, 5, 6, 7. Different stages, showing the gradual shortening of the chromosomes in some cases, in others different forms of chromosomes, in same stage as those in *fig. 3*. In *figs. 4* and *5*, some are opening out to form rings; in *fig. 6* one of the oblong rings is twisted to form a figure 8, and in *fig. 7* one shows two openings along the line of longitudinal division. All of the chromosomes in these figures are yet more or less irregular, angular, and show fragments of the linin attached.

FIGS. 8, 9, 10. Chromosomes much shortened, and a large number of them in the ring form. In *fig. 8* two have completely divided to form rods, two form oblong plates with forked ends, and one forms a ring, while in *fig. 10* nearly all are in the ring form. Some of them are still angular, and show the peculiar form of so many of the rings of Arisæma. The tetrads are being formed by the accumulation of the chromatin in denser portions near the ends of the rods, and near the ends of each half of the rings, the paler zone across the middle showing the line of transverse division. Linin threads are still attached to some of the angles. The nuclear membrane is still intact, and the chromosomes are arranged around the periphery of the nuclear cavity in the positions occupied by them when they separated as segments from the spirem.

FIGS. 11, 12, 13. The nuclear membrane has disappeared, and threads of kinoplasm are entering to form the spindle; chromosomes in the form of rings, paired rods, or angular plates, which are solid or with a central perforation indicating the ring form.

FIG. 14. Spindle with chromosomes approaching the nuclear plate. The poles of the spindle show radiating lines of protoplasm indicating centers of force.

FIG. 15. Chromosomes in the nuclear plate stage, but they are in this preparation so fused that it is impossible to see the way in which division takes place.

FIGS. 16, 17. Chromosomes beginning to separate at the nuclear plate; rings, paired rods, and tetrads can be seen. The rings and paired rods are lying with the long axis parallel with the axis of the spindle, longitudinal division has taken place in some and is taking place in others, so that the halves of rings form crescent-shaped rods, the tetrads are indicated by deeper staining portions of the rods or rings.

FIGS. 18, 19. A little later condition of the same (metaphase) stage; longitudinal division has taken place so that nearly all the rods or halves of the rings have separated, and by the pulling of the spindle threads the tetrads are being drawn apart transversely to the axis of the chromosome, to bring about the reducing division.

FIGS. 20, 21. Polar view of the metaphase, showing that the chromosomes are distributed all through the nuclear plate. Sixteen groups can be counted, bearing in mind that a pair of rods, or a tetrad, makes a group. Some of the chromosomes are turned somewhat obliquely, especially in *fig. 20*, which was an oblique view of the plate, and in some cases the paired rods are seen only from the end.

FIG. 22. The tetrads have nearly separated by transverse division.

FIGS. 23, 24. Tetrads, about 32 on each side, approaching the poles during the anaphase of the first mitosis.

PLATES III–VI. Trillium grandiflorum.

FIG. 1. Portion of the spirem with partial longitudinal division, and a few chromosomes.

FIGS. 2, 3, 4, 5. Early stage in the formation of the chromosomes just after the segmentation of the spirem, showing, in some, openings along the middle line, in others the ends forked, indicating the line of longitudinal division. Pairs of deeply staining chromatin masses are evident in some of the chromosomes, frequently four pairs, or eight masses in a single chromosome.

FIGS. 6, 7, 8, 9, 10. The nuclear membrane has disappeared, and the chromosomes are being drawn up to the nuclear plate.

FIGS. 11–17. Various figures showing the metaphase stage, the orientation of the chromosomes in the nuclear plate; in some, as in *fig. 11* and *17*, the four paired masses of denser chromatin are well seen; in *fig. 17* the pull of the spindle threads is drawing out the middle portion on either side of the broad chromosome, the first step in the separation of the longitudinally divided segment.

FIGS. 18, 19. Two different stages in the separation of the chromosomes in the V form as they are pulled toward the poles.

FIG. 20. Cell plate formed after first mitosis, and U or horseshoe-shaped chromosomes lying in position as they approached the poles.

FIGS. 21, 22. Oblique view of two poles, to show the position of the chromosomes as they come to the poles; in *fig. 21* the group has already elongated somewhat in the direction of the axis of the spindle for the second mitosis.

FIGS. 23, 24, 25, 26. Position of the chromosomes in the daughter nucleus after first mitosis; nuclear membrane formed.

FIG. 27. Nuclear membrane has disappeared at the prophase of the second mitosis, and the chromosomes, still showing the form and characters present when reaching the poles after the first mitosis, are beginning to move to the nuclear plate of the second mitosis.

FIGS. 28, 29, 30. Later stages as the chromosomes are approaching the nuclear plate. The U form is closing up by the folding of the arms, and in some, transverse division has taken place so that they form a pair of rods; in a few the ends of the arms have met and form a ring, which simulates the form of chromosomes in the heterotypic division.

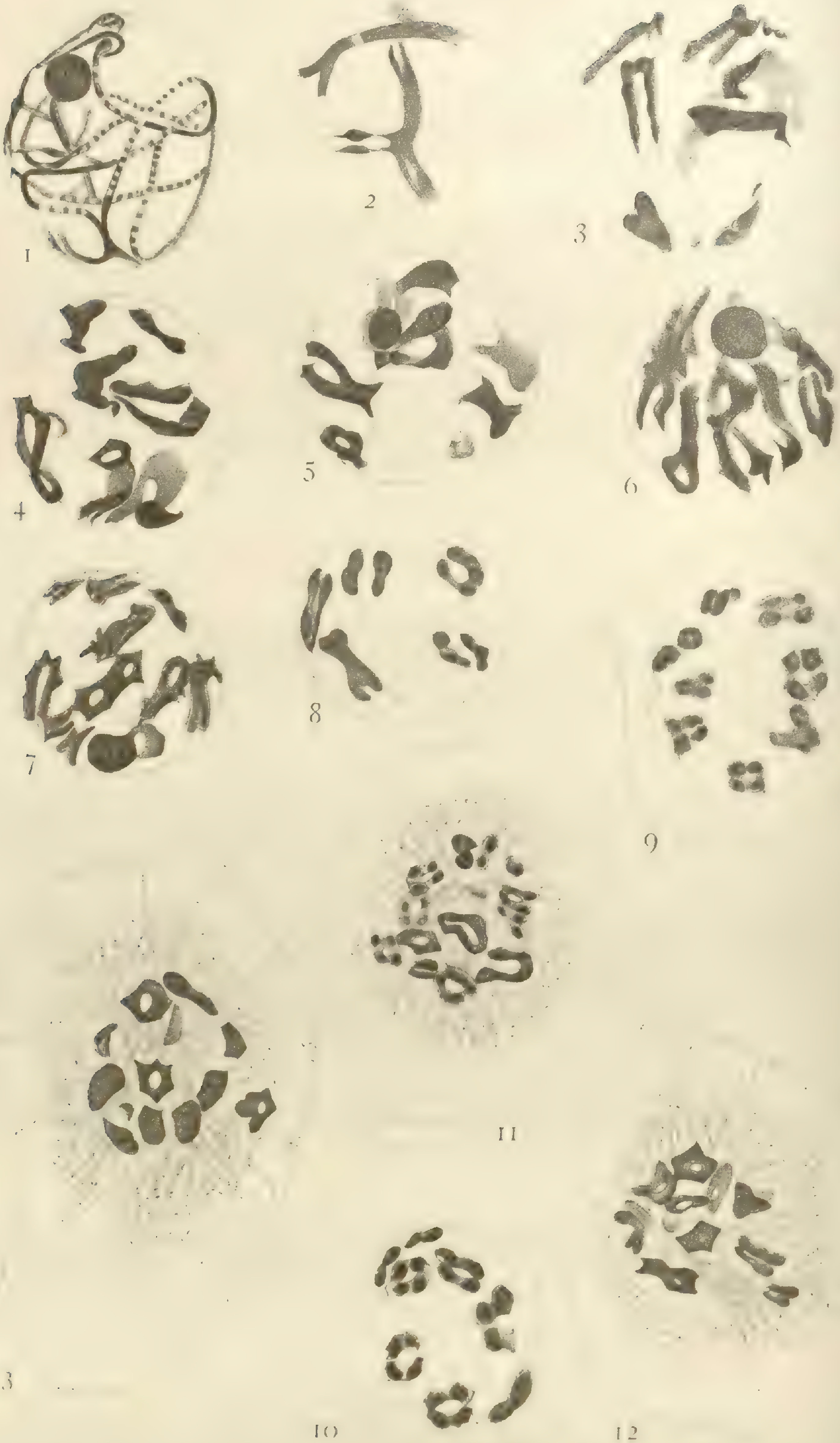
FIG. 31. End view of nuclear plate stage in second mitosis.

FIG. 32. Side view with spindle established, the chromosomes presenting much the same form as in the first mitosis, from the closing up of the arms of the U and the cross division at the junction of the arms.

FIGS. 33, 34, 35. Different stages in the separation of the transversely divided chromosomes at the nuclear plate in second mitosis.

FIG. 36. Three different forms of the chromosomes as they are passing to the poles in the second mitosis; the V form; hooked, or J form; and the rod form, depending on the point of attachment of the spindle threads; all in the same nucleus.

FIGS. 38, 39. End view of spindle showing chromosomes approaching the poles at the close of the second mitosis.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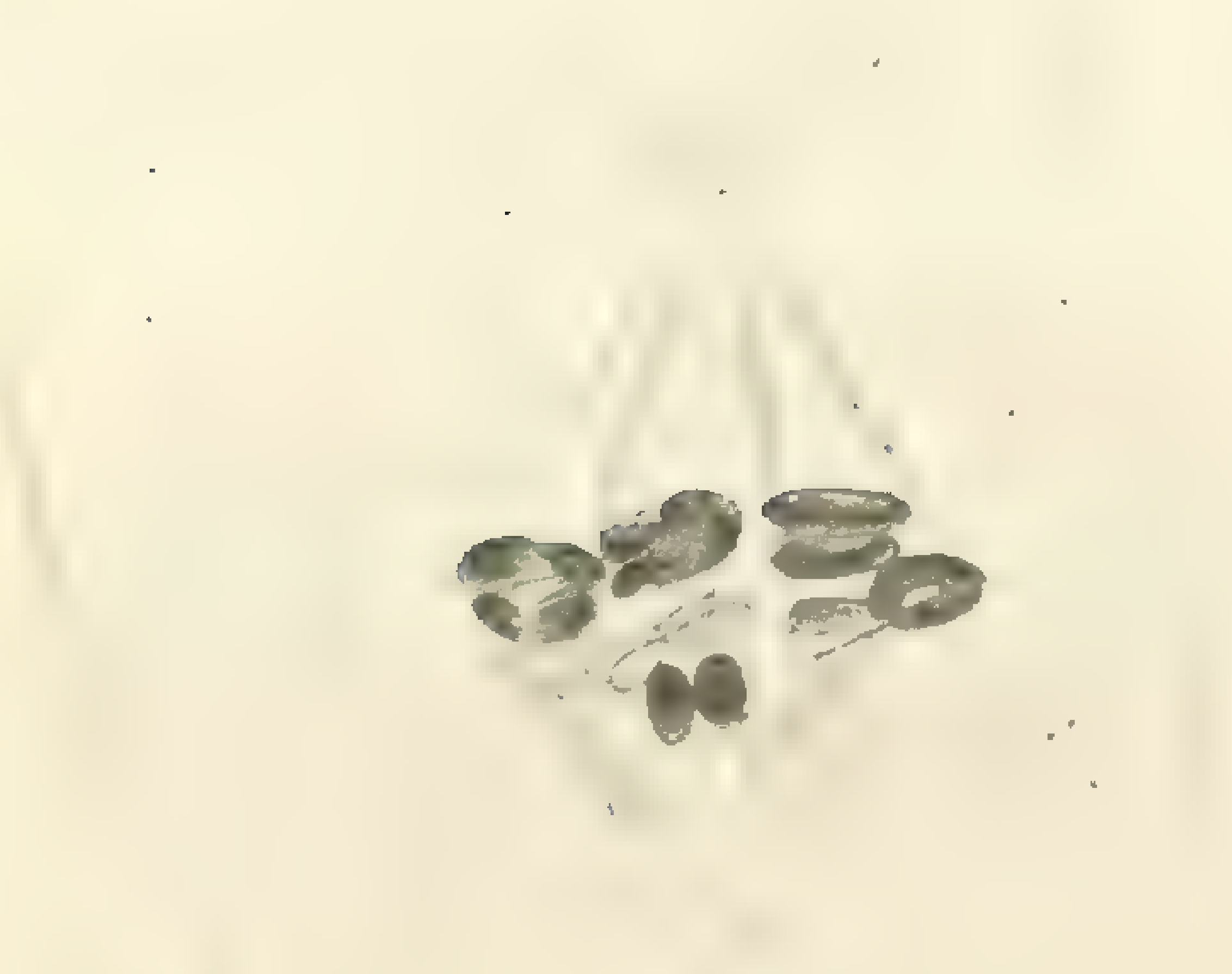

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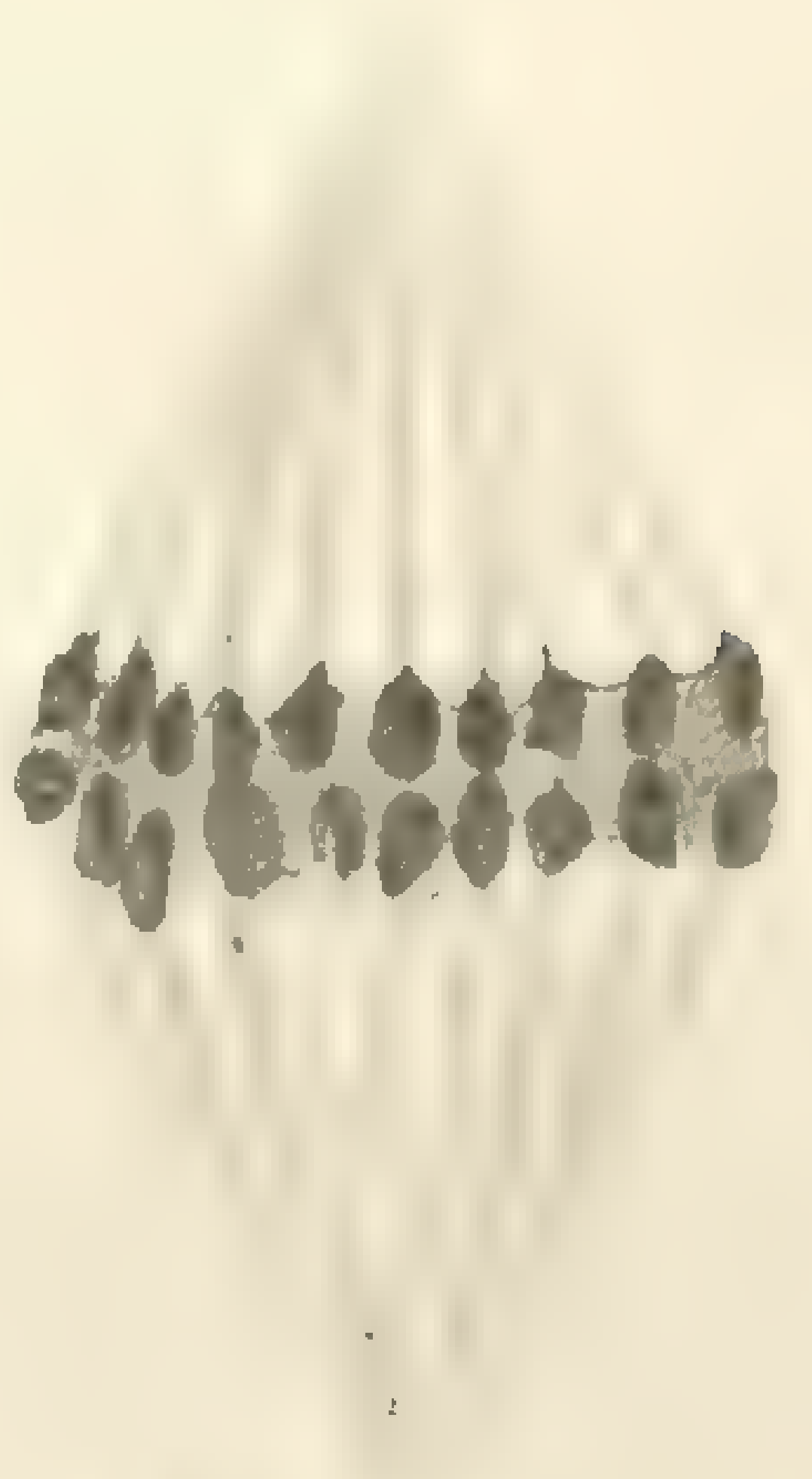

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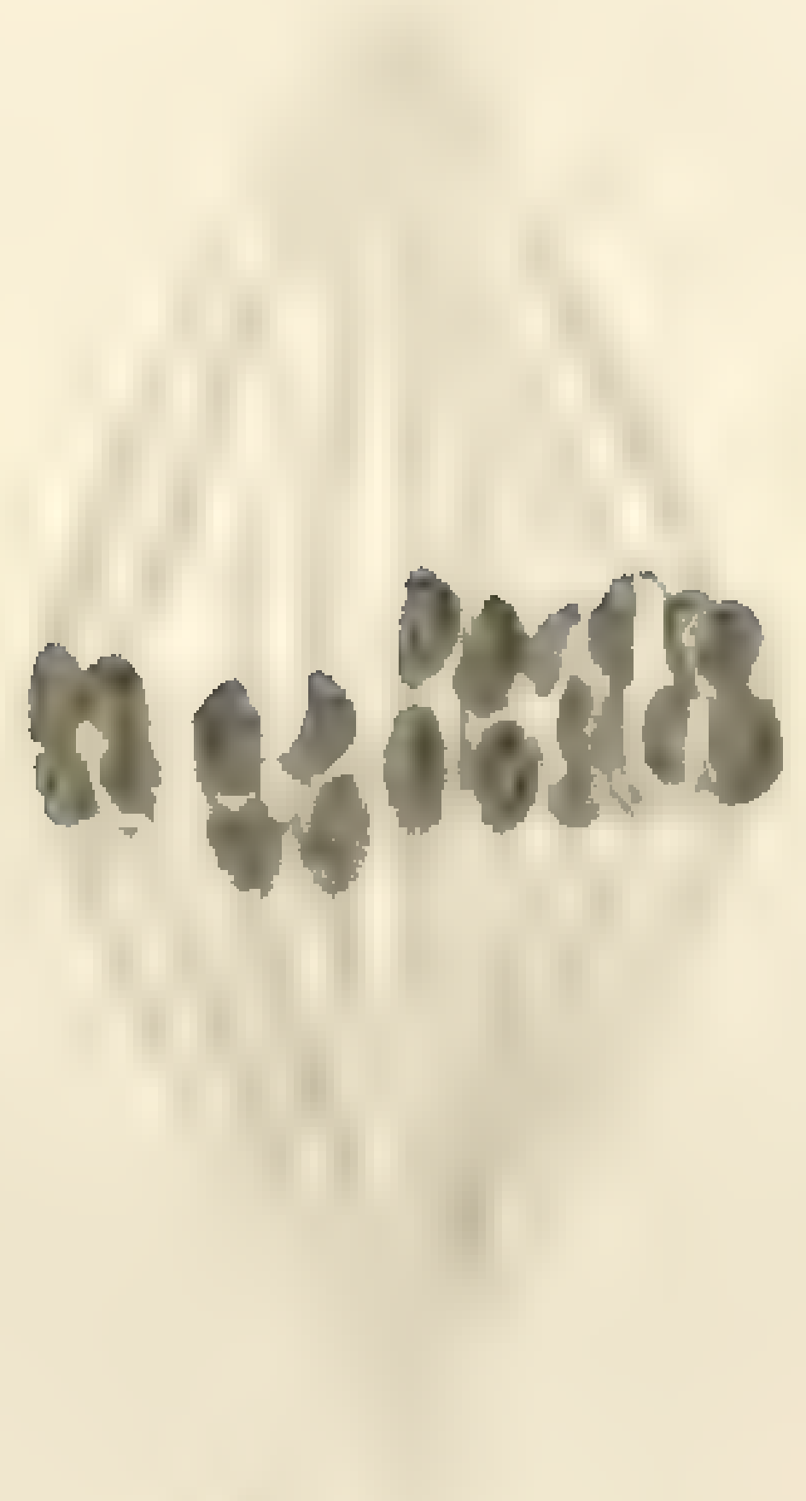

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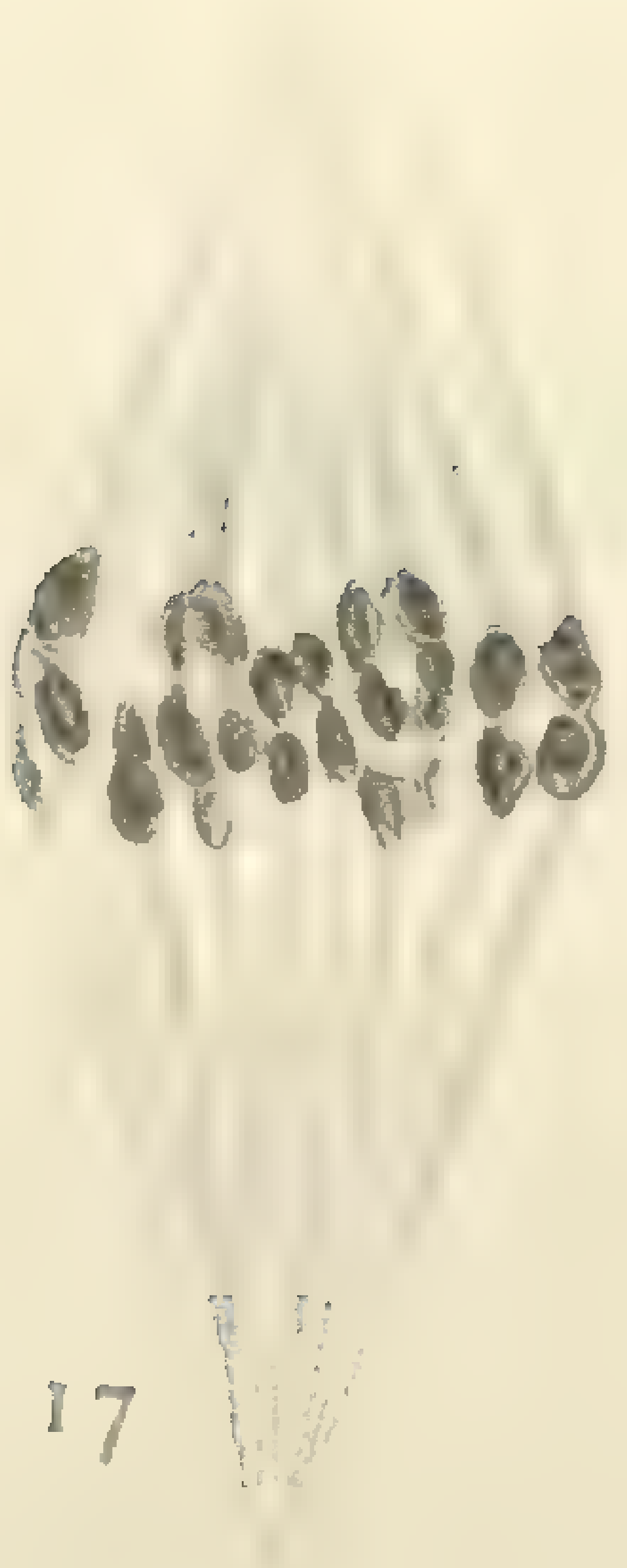

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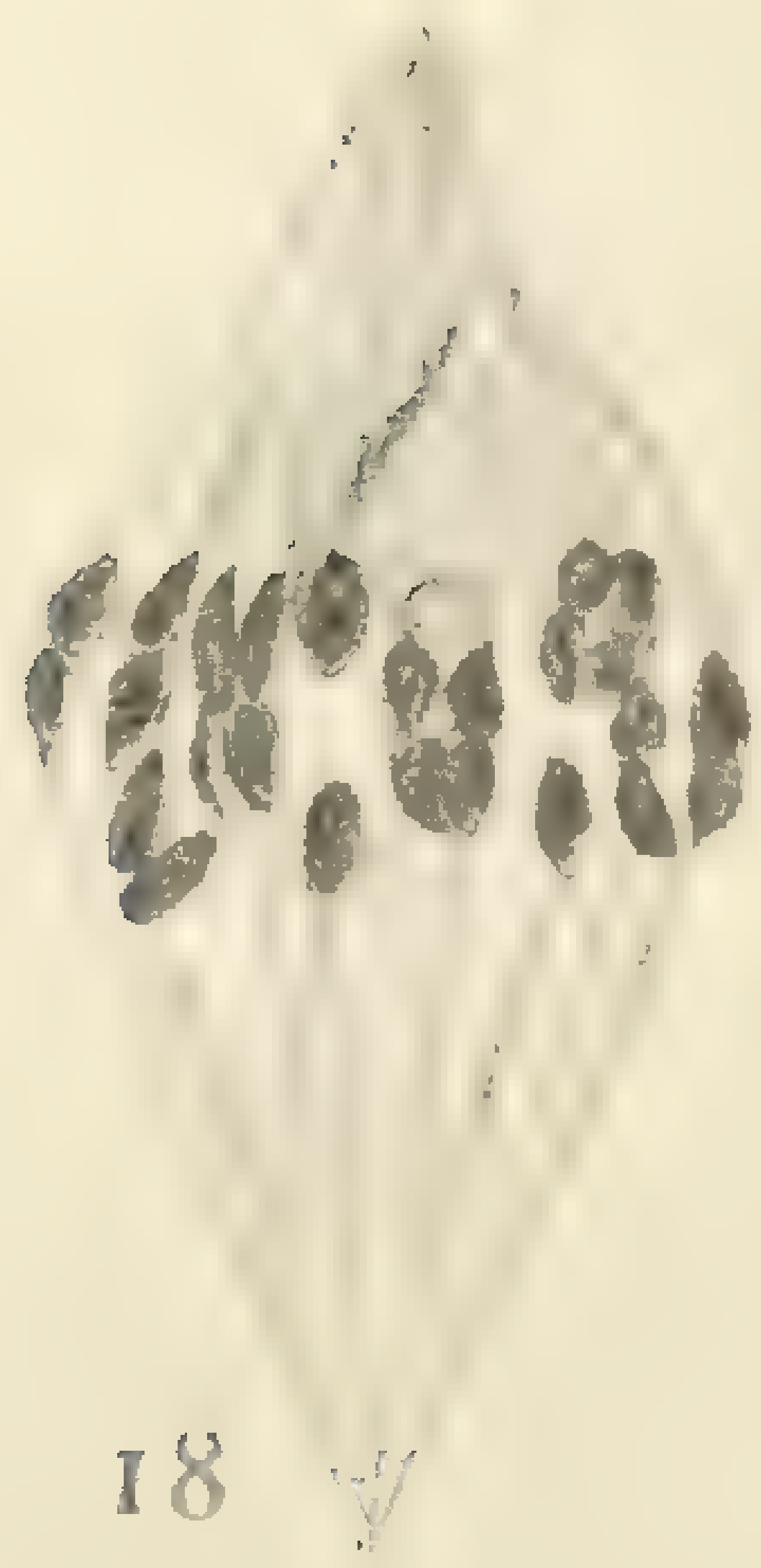

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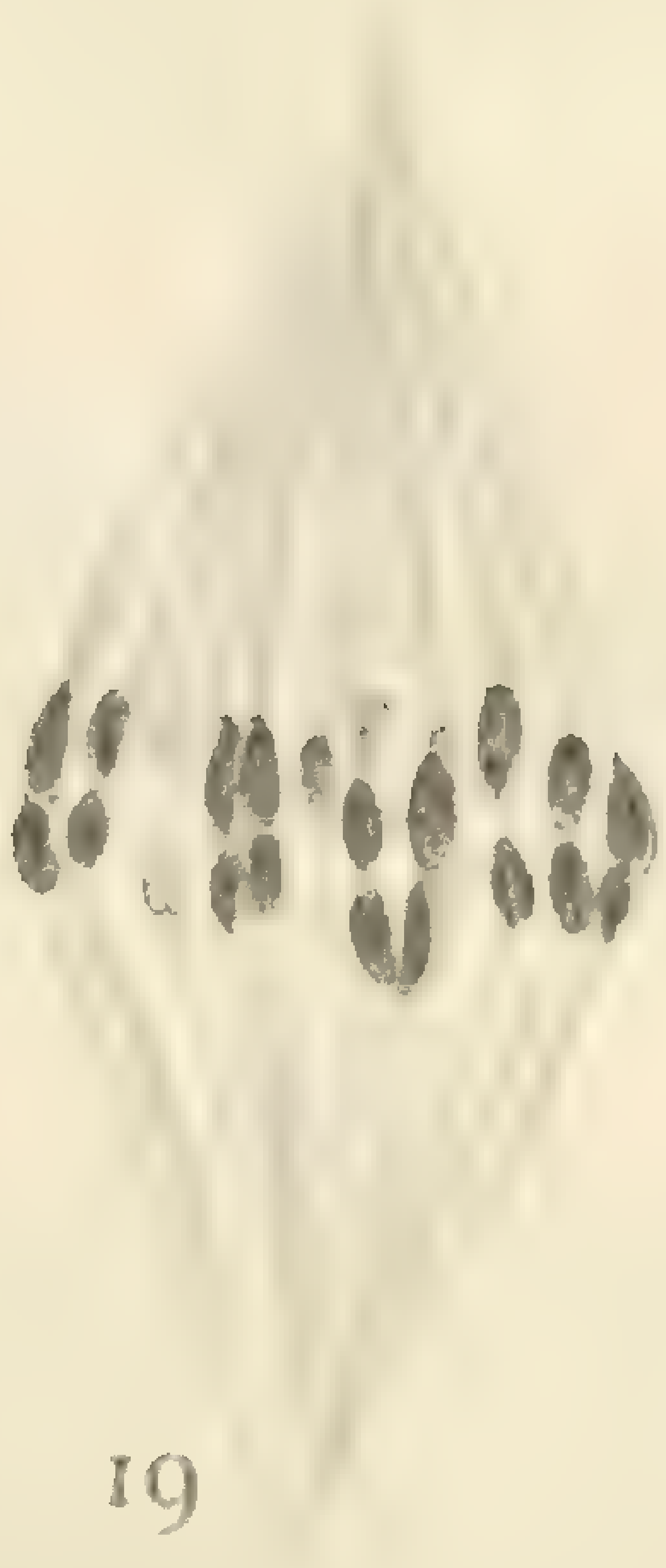

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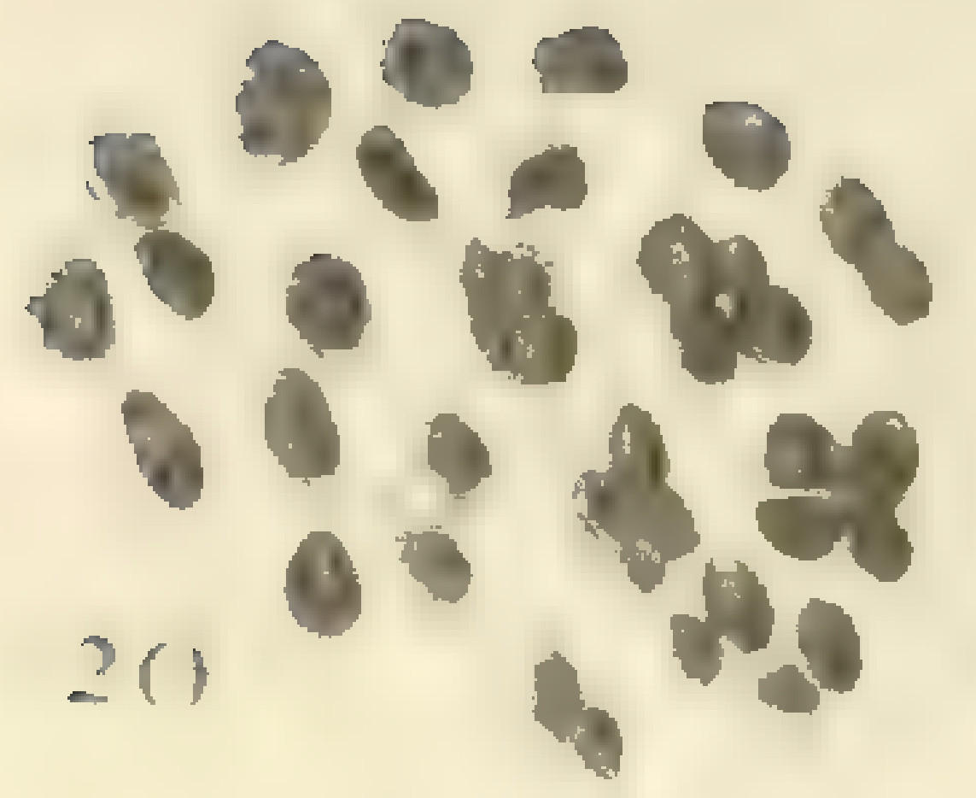

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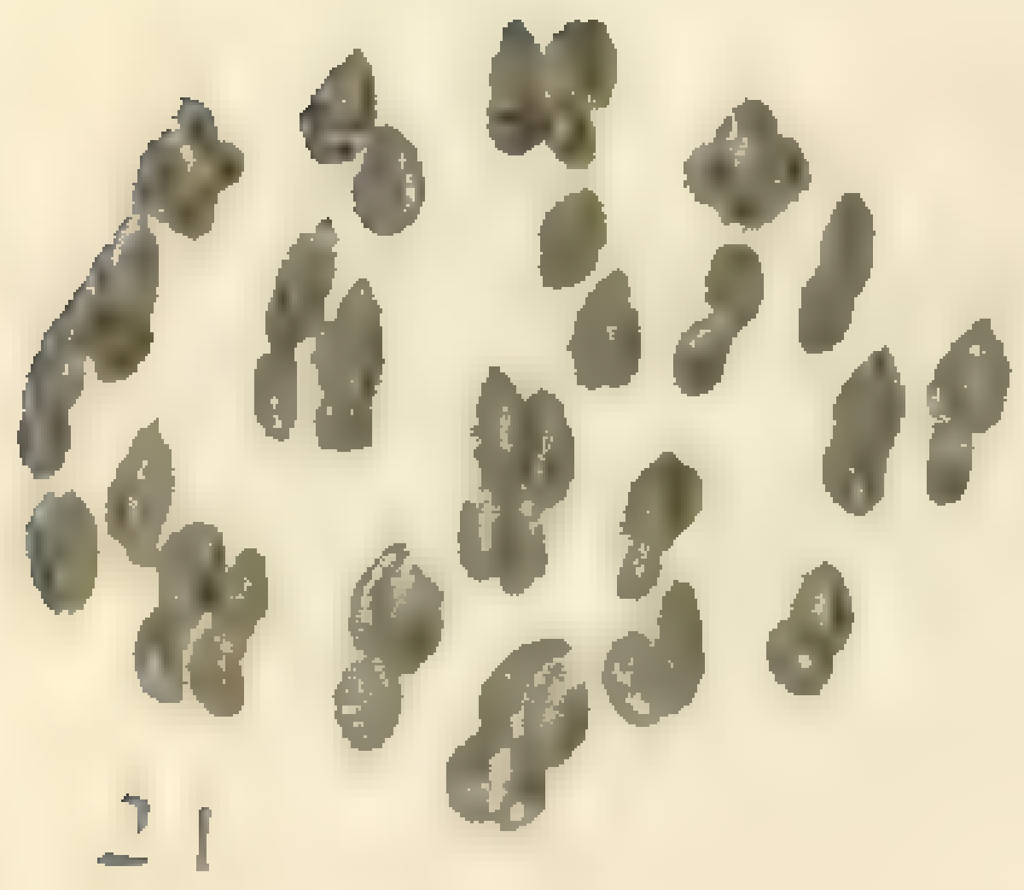

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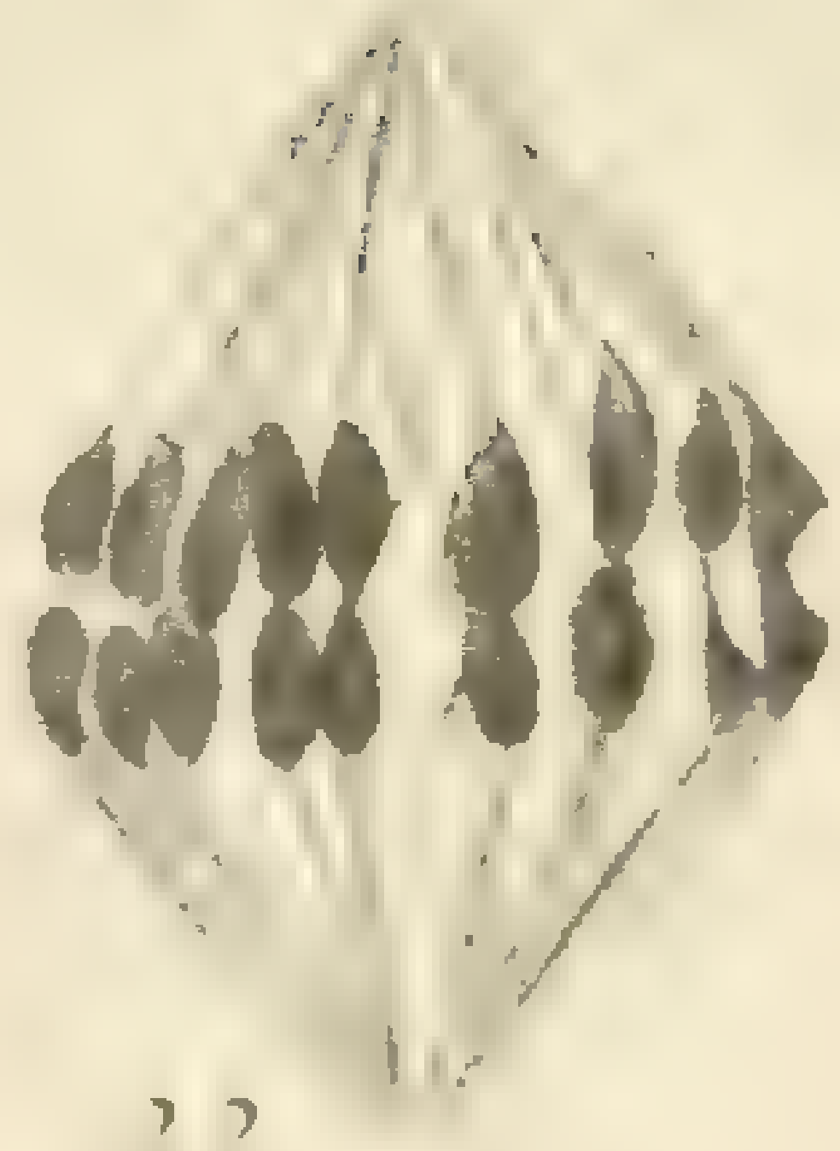

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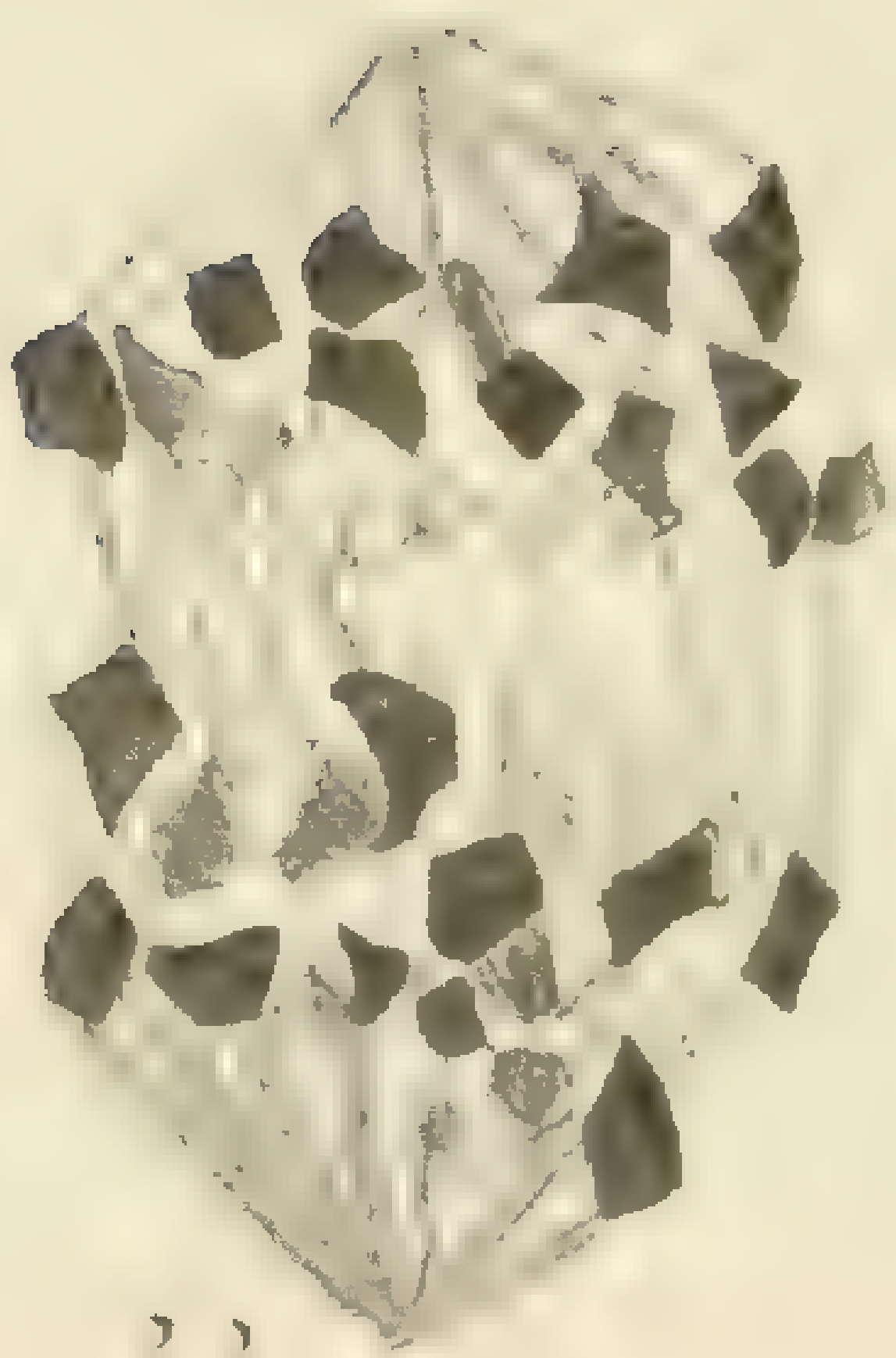

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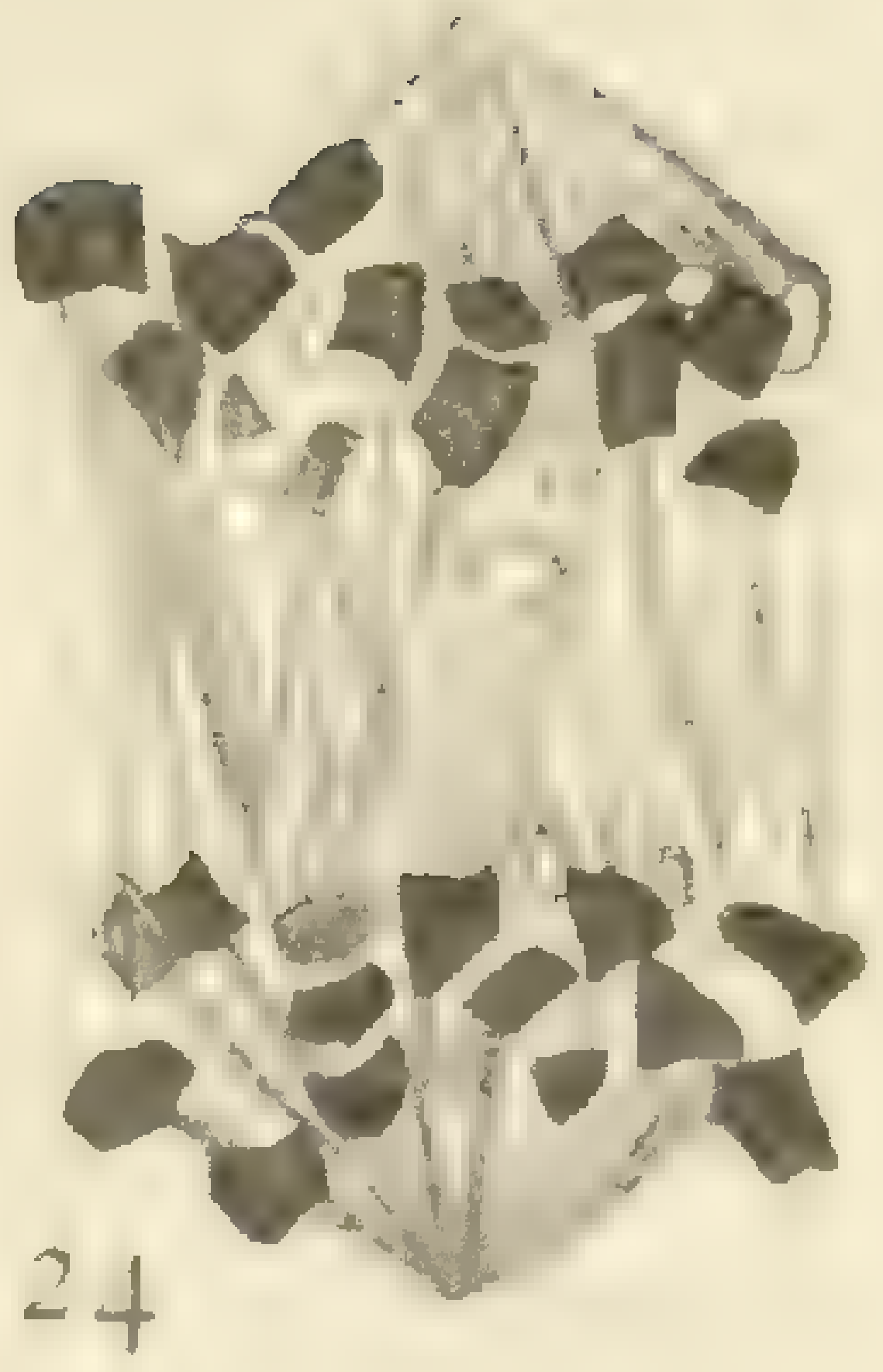

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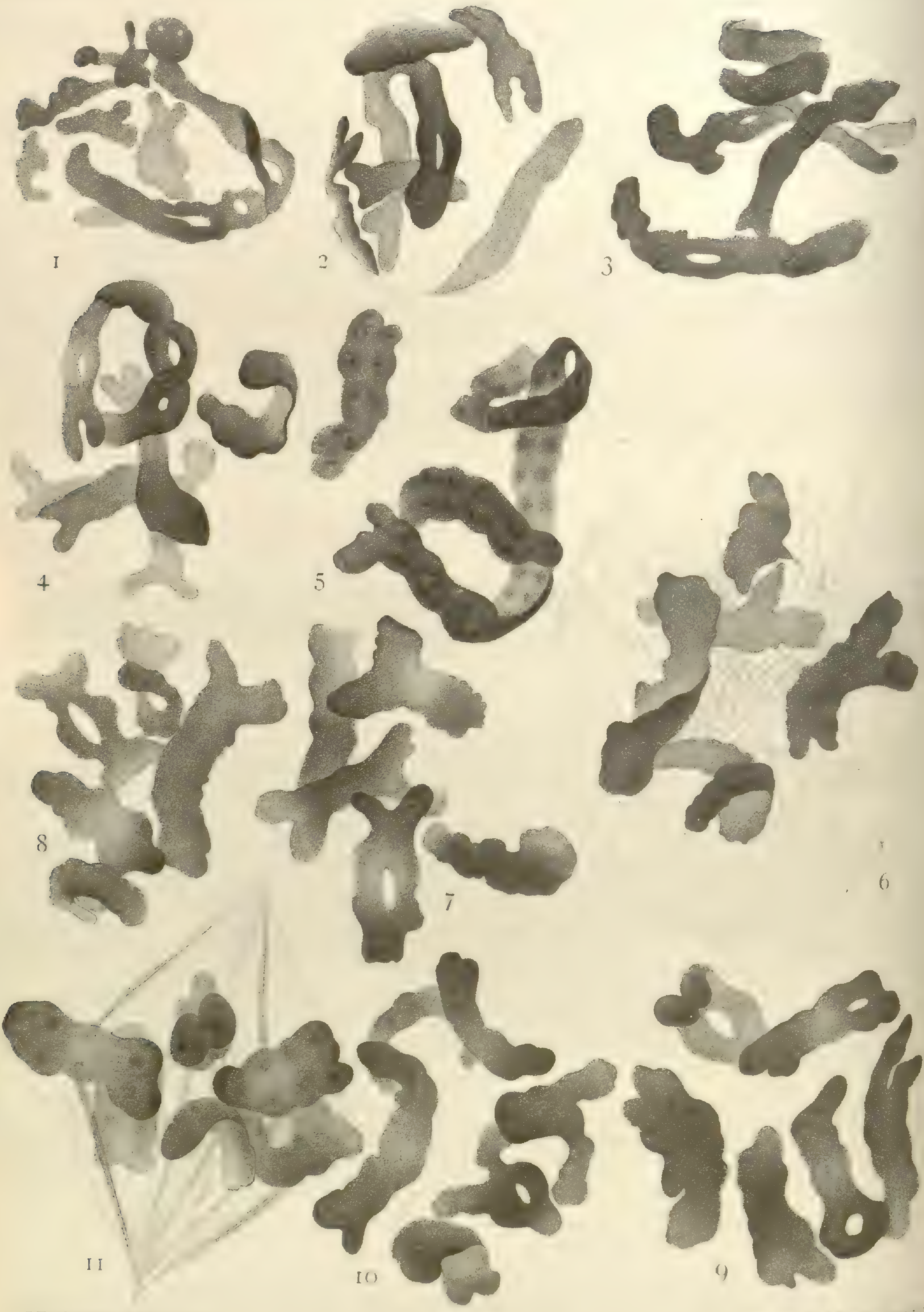
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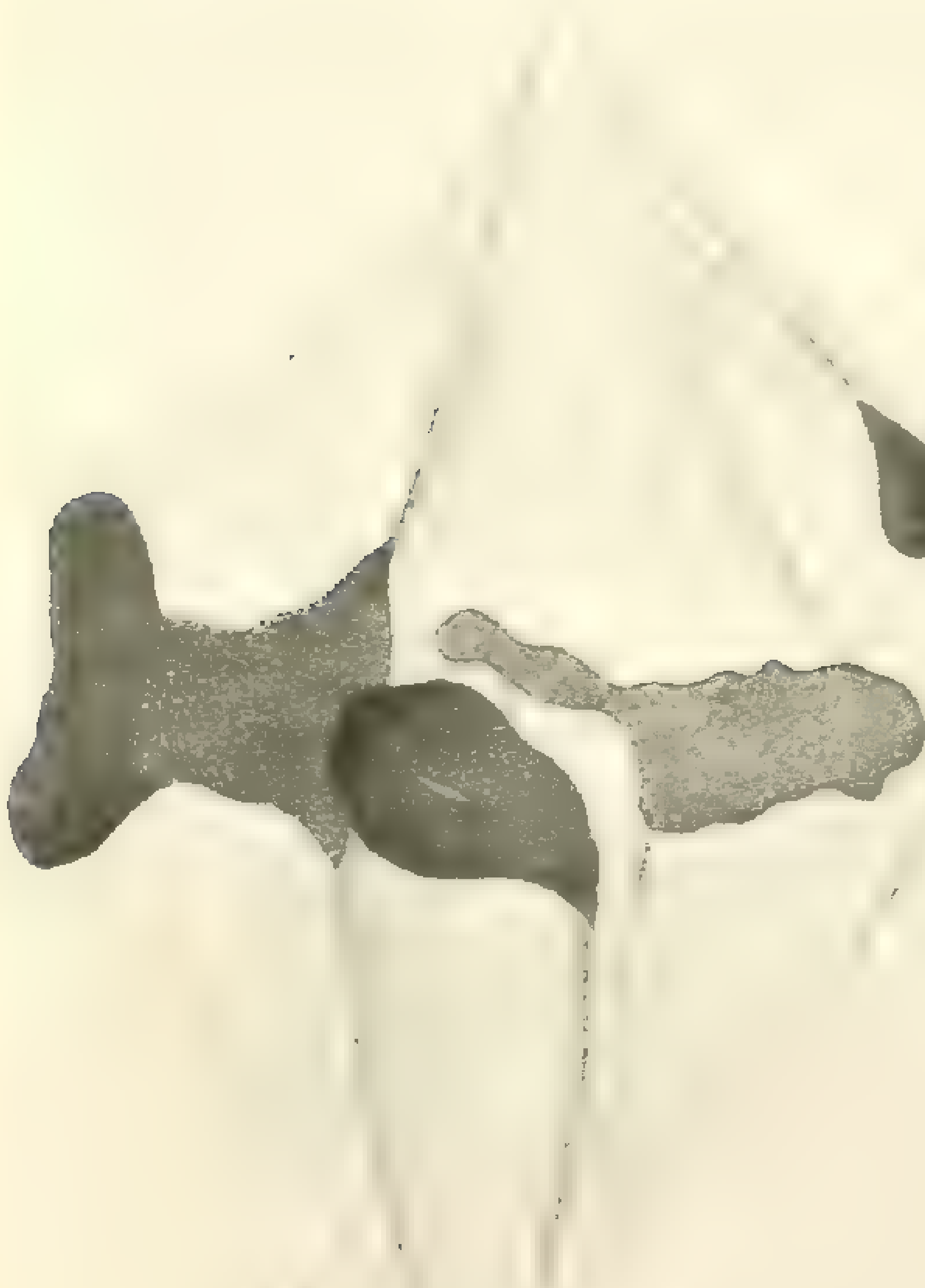

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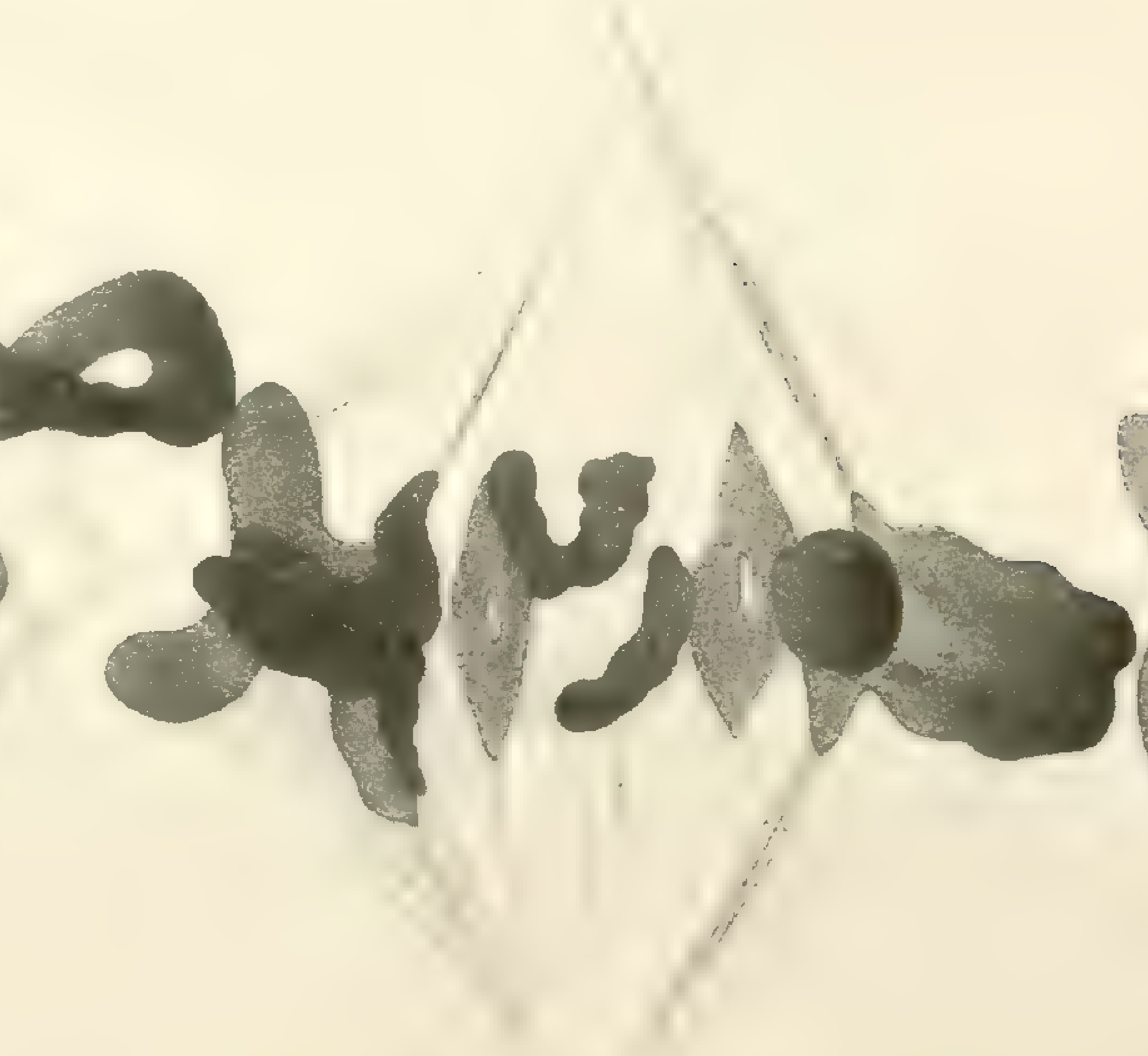

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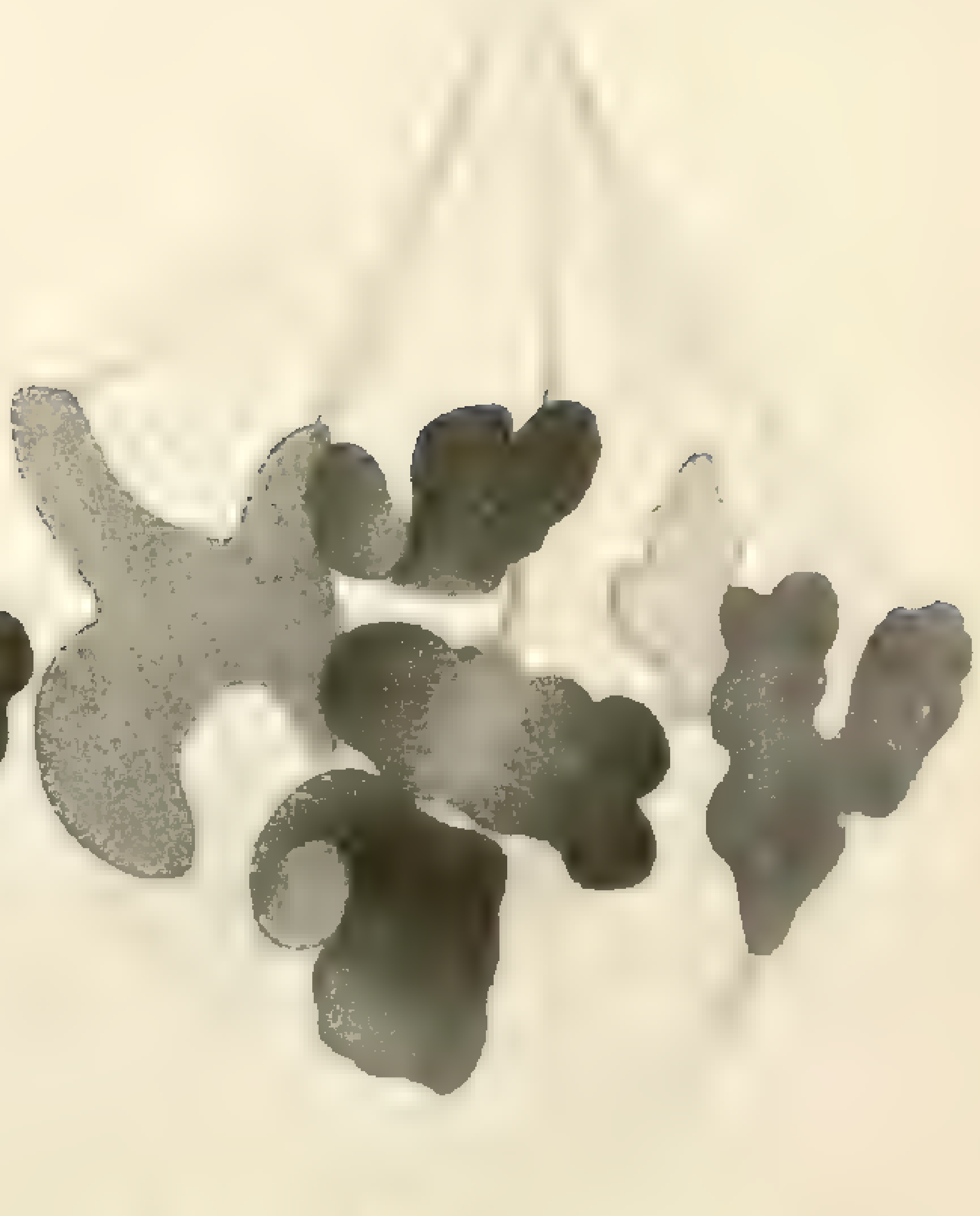

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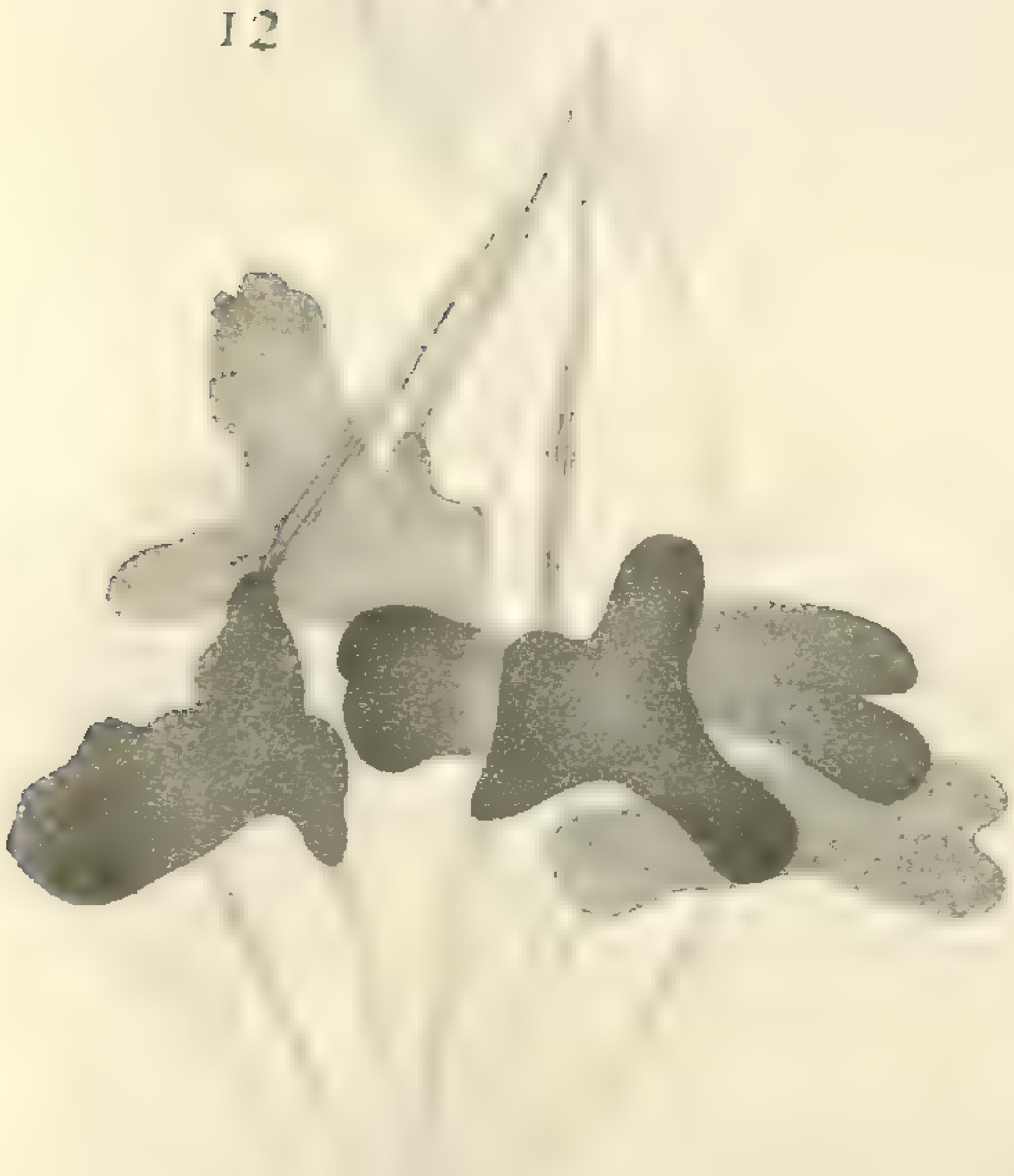

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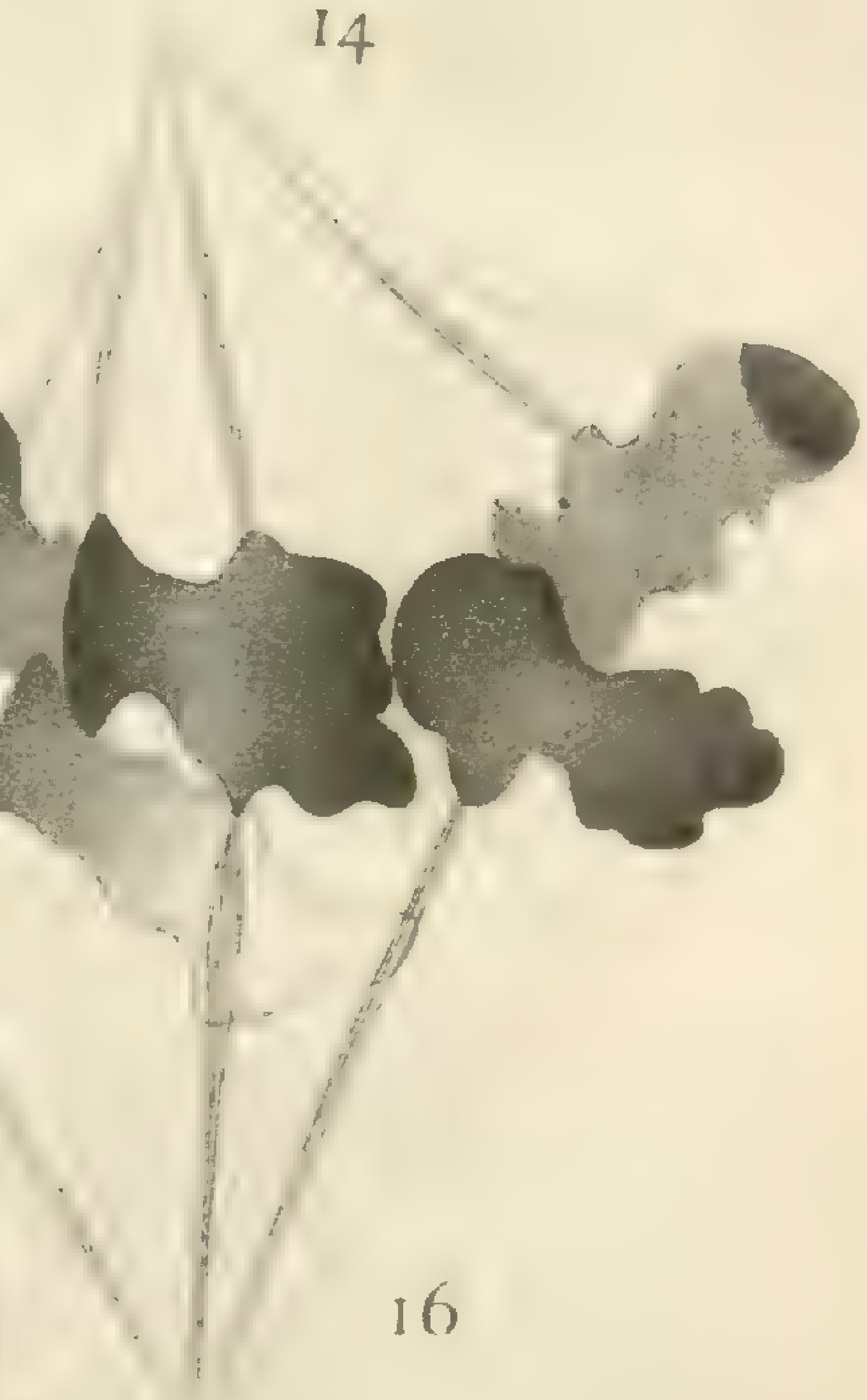

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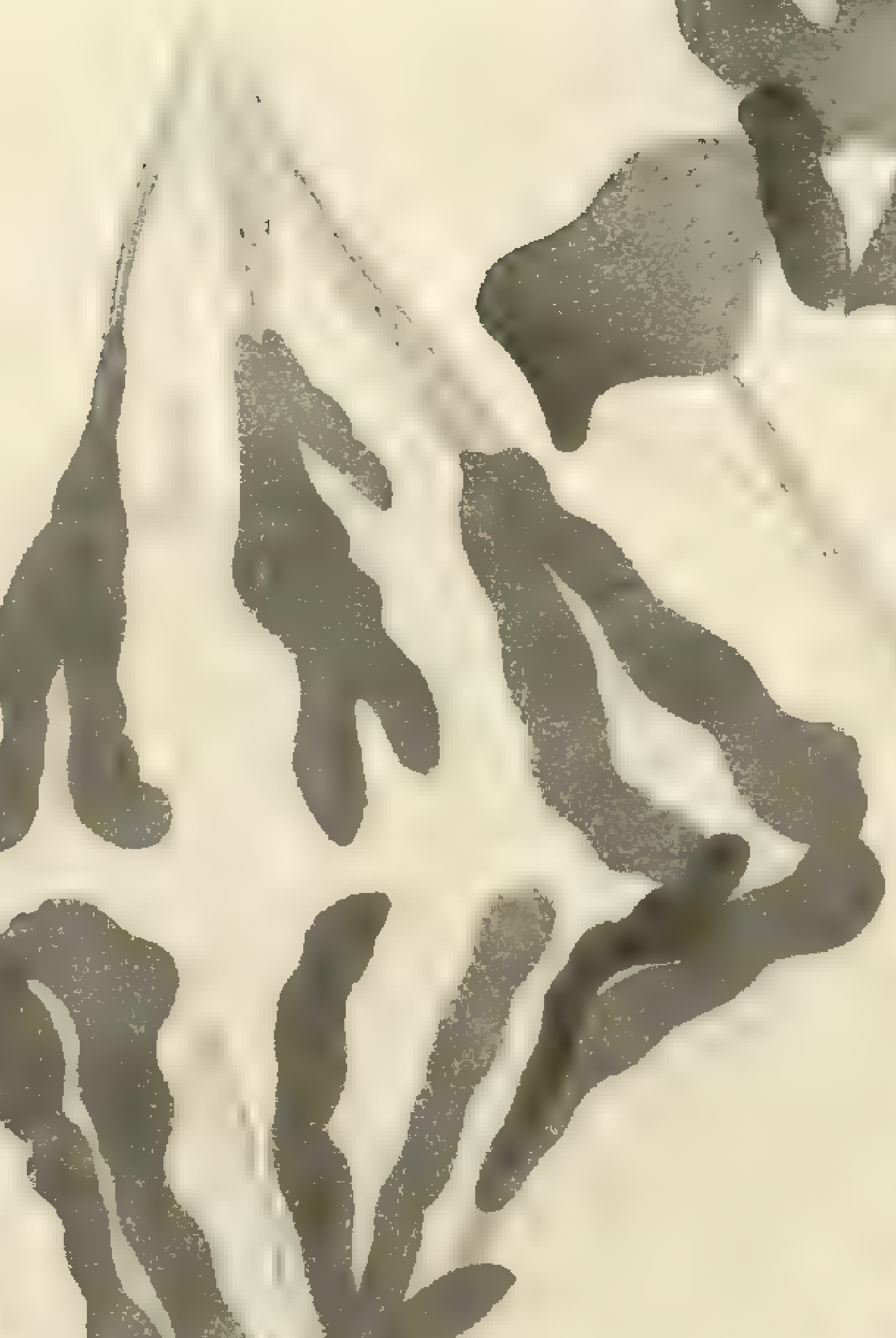

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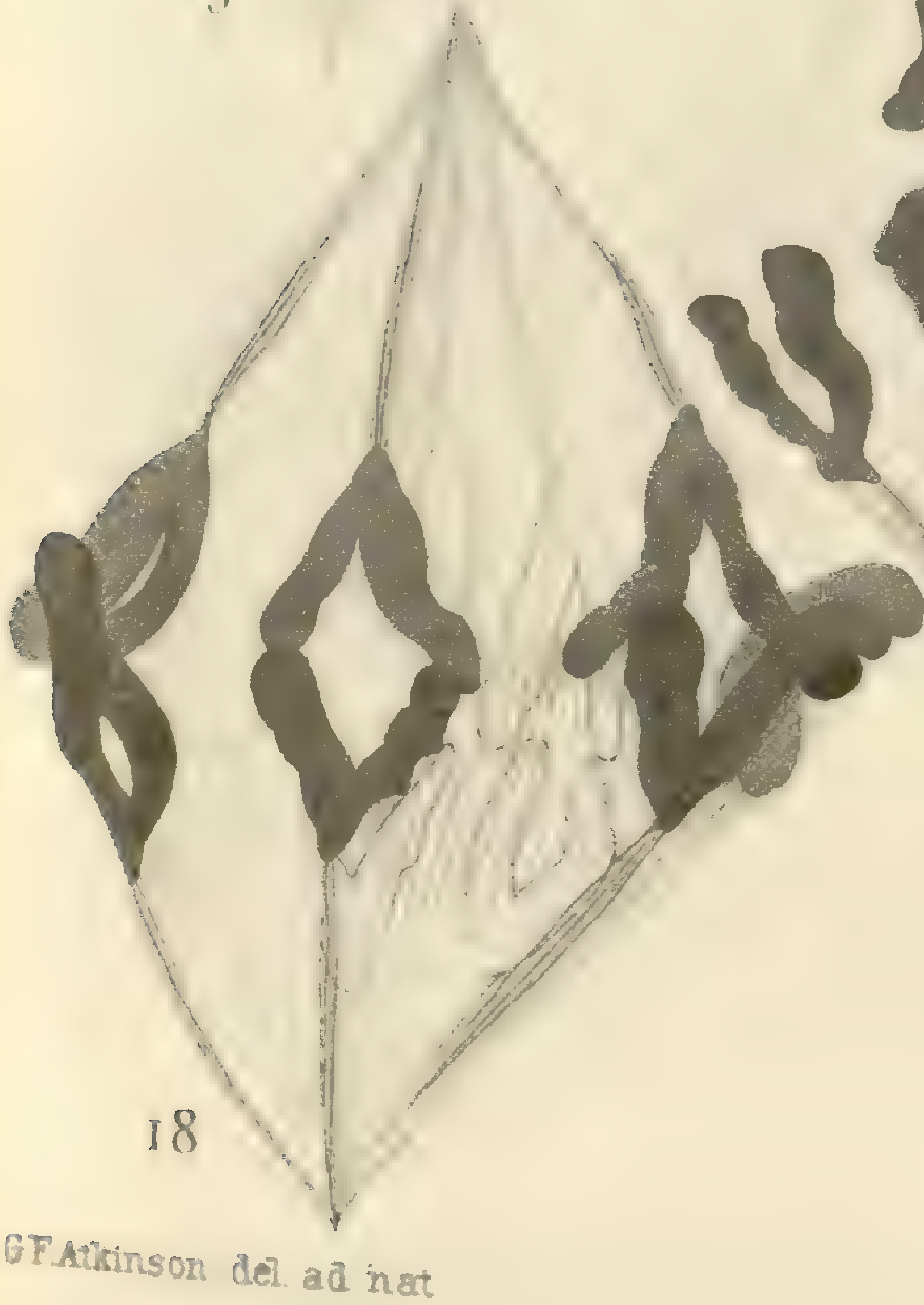

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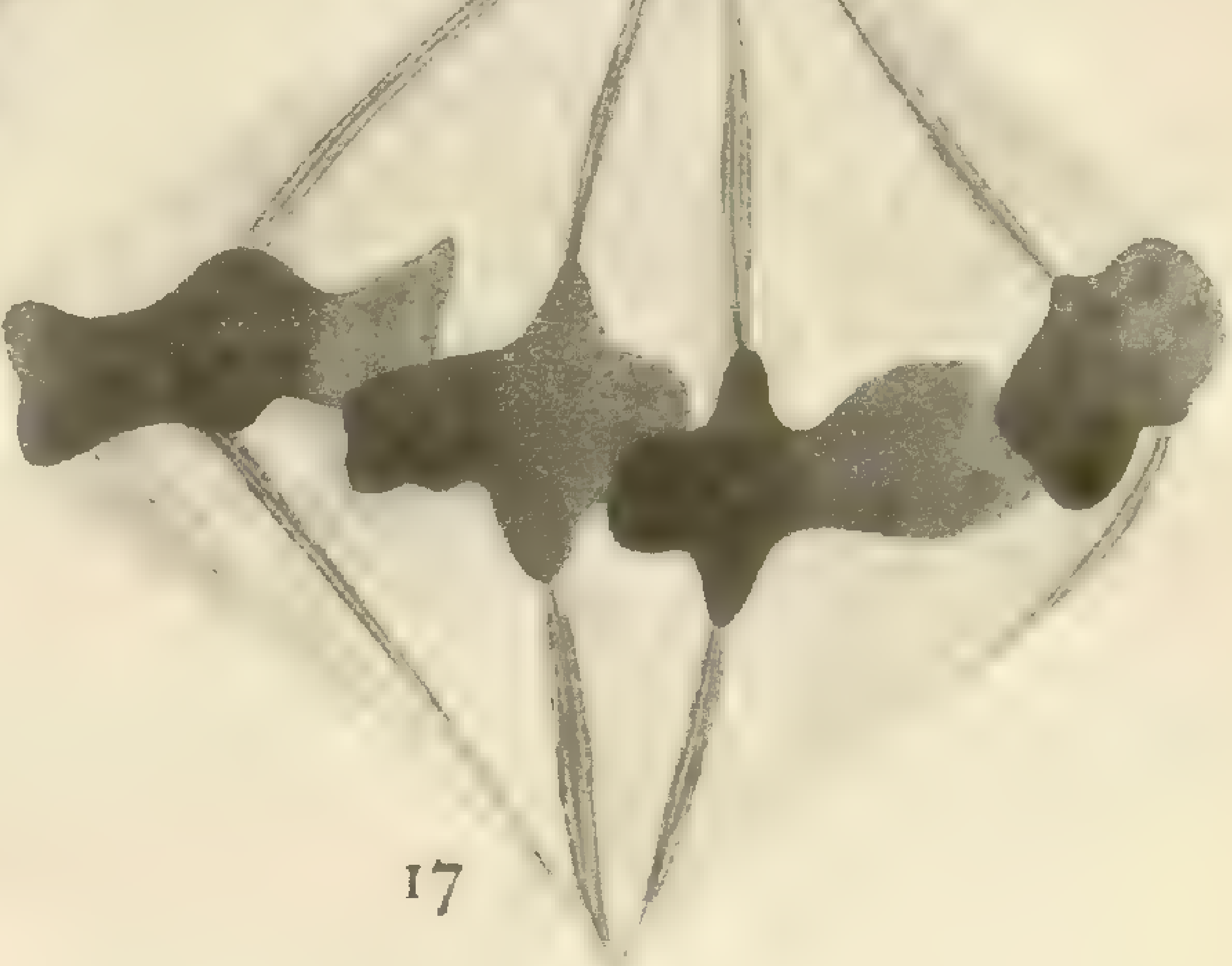

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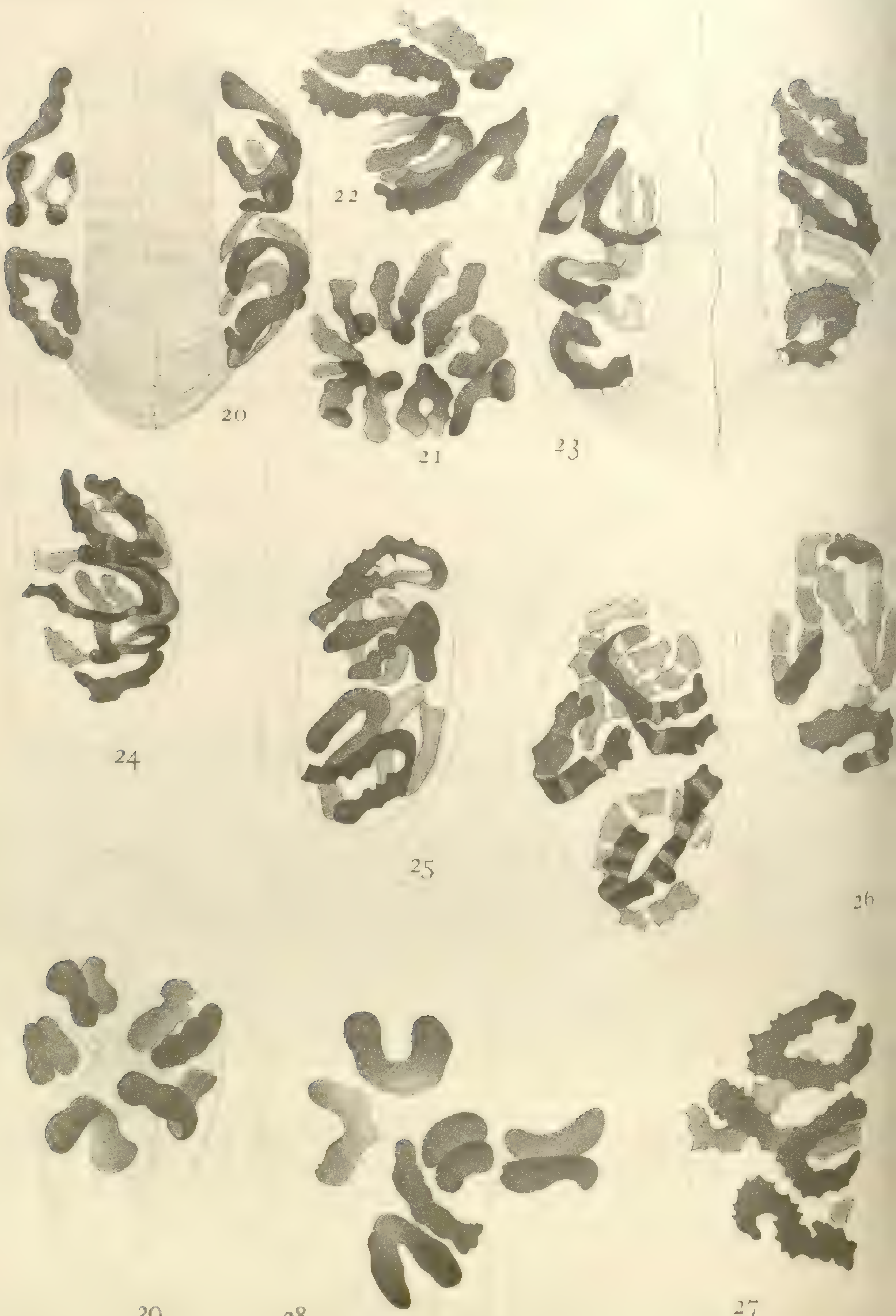
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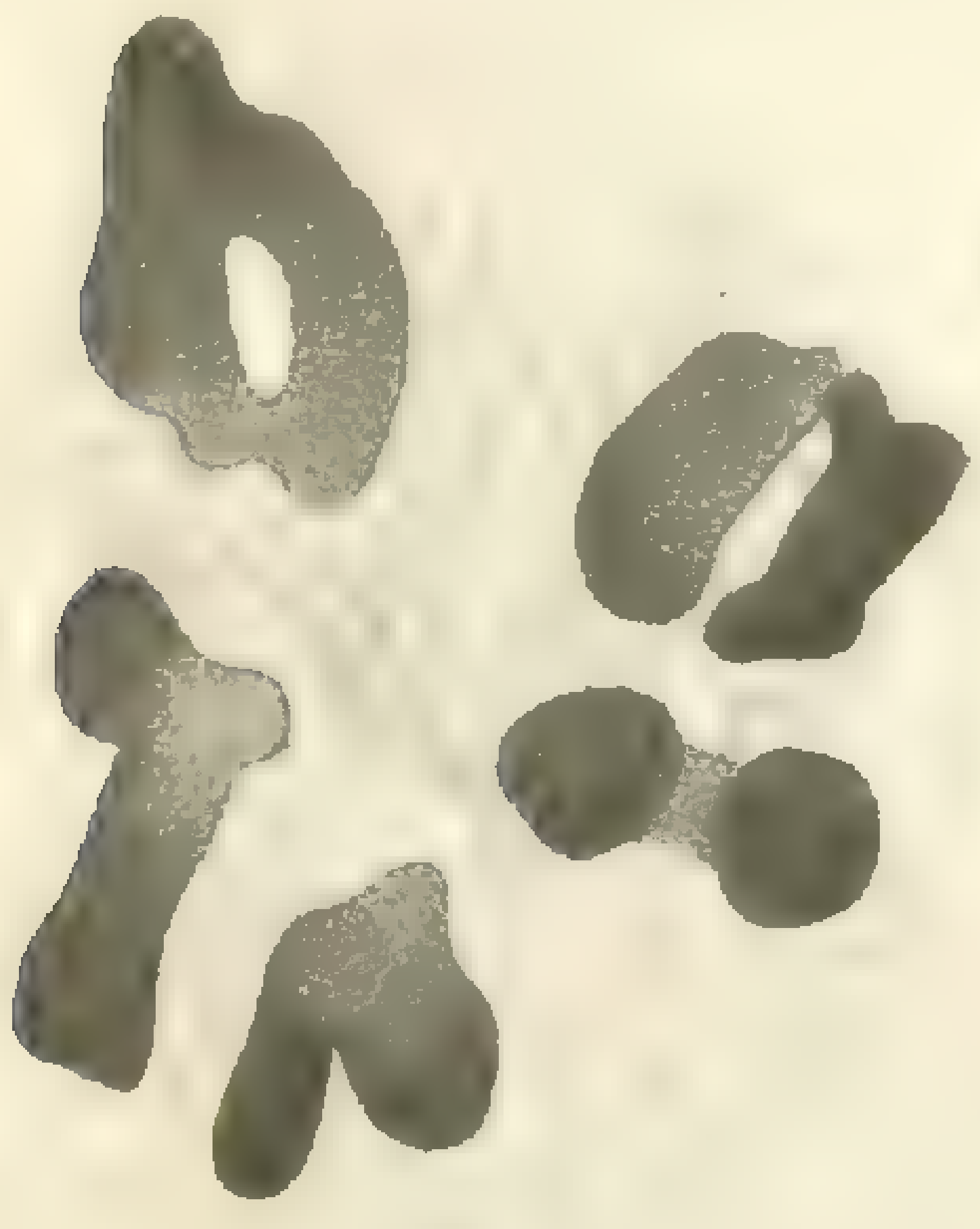
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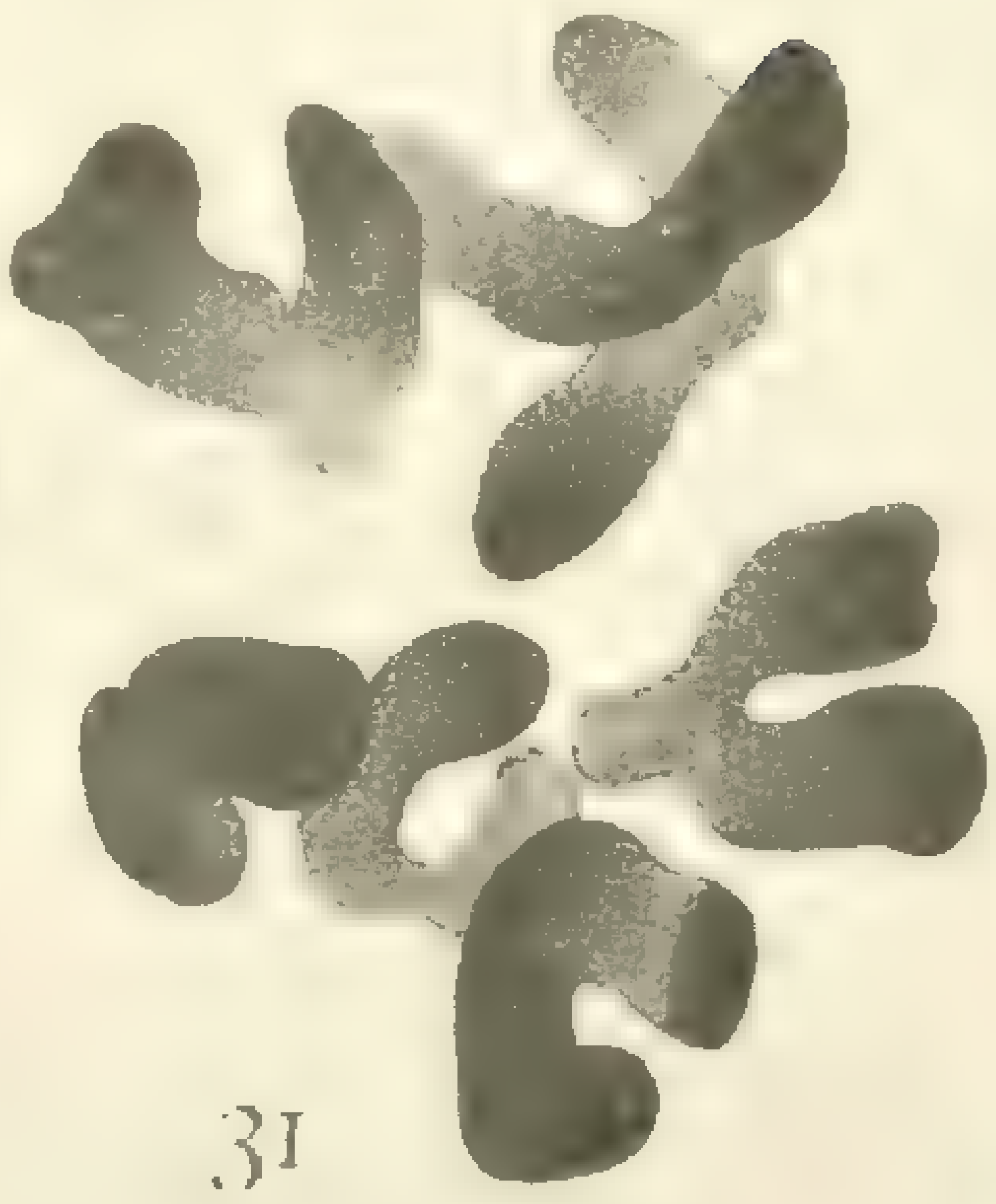
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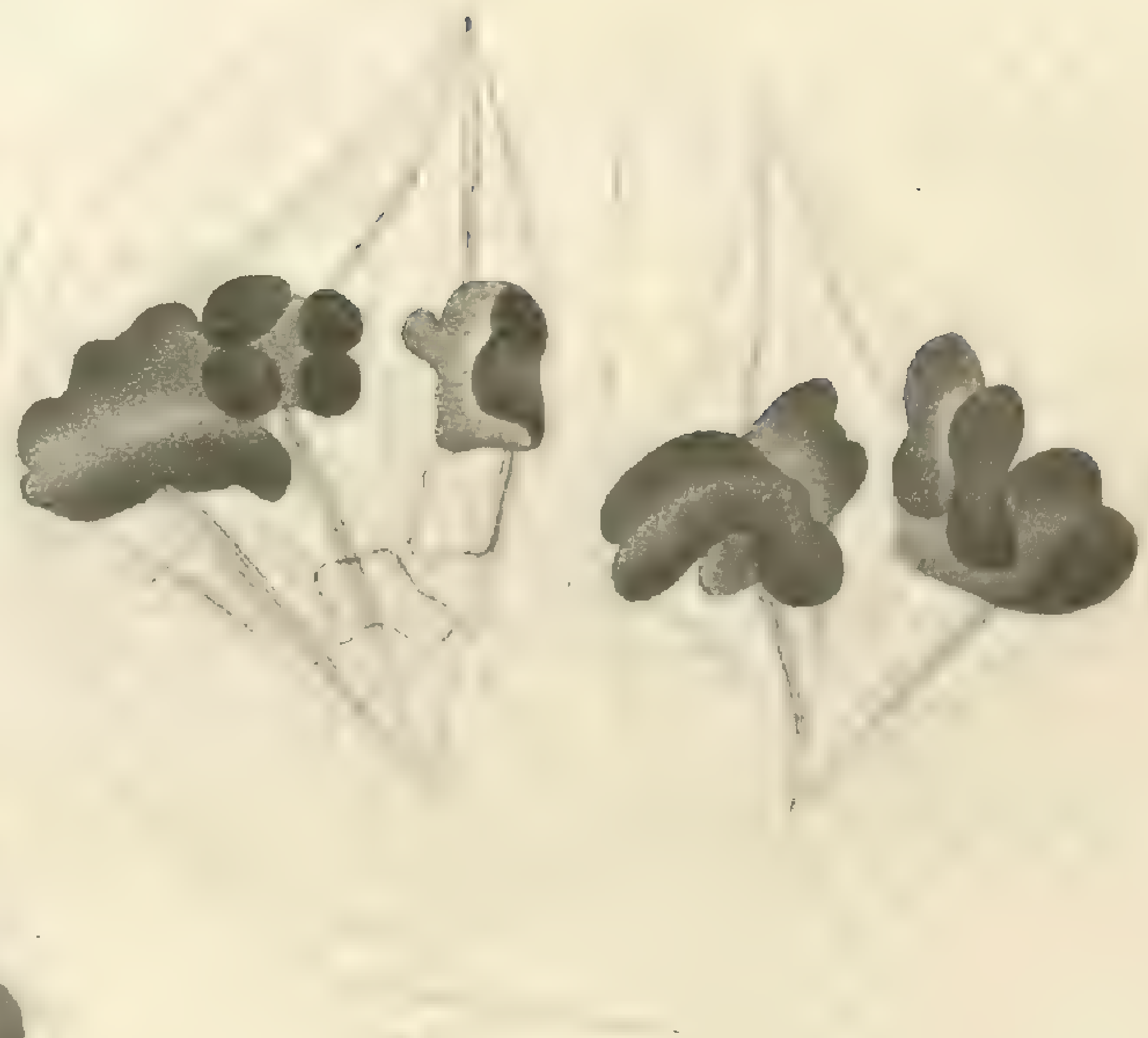
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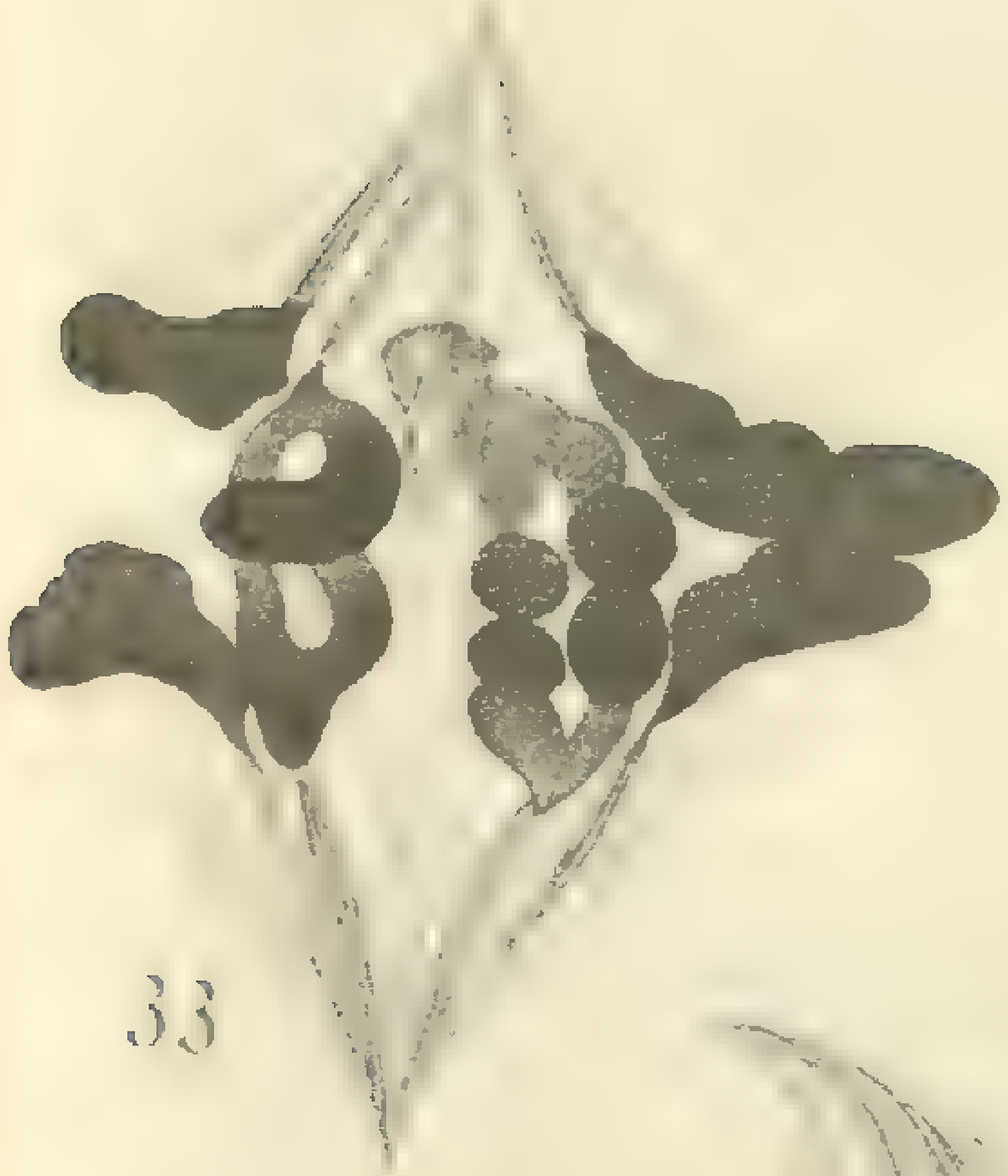
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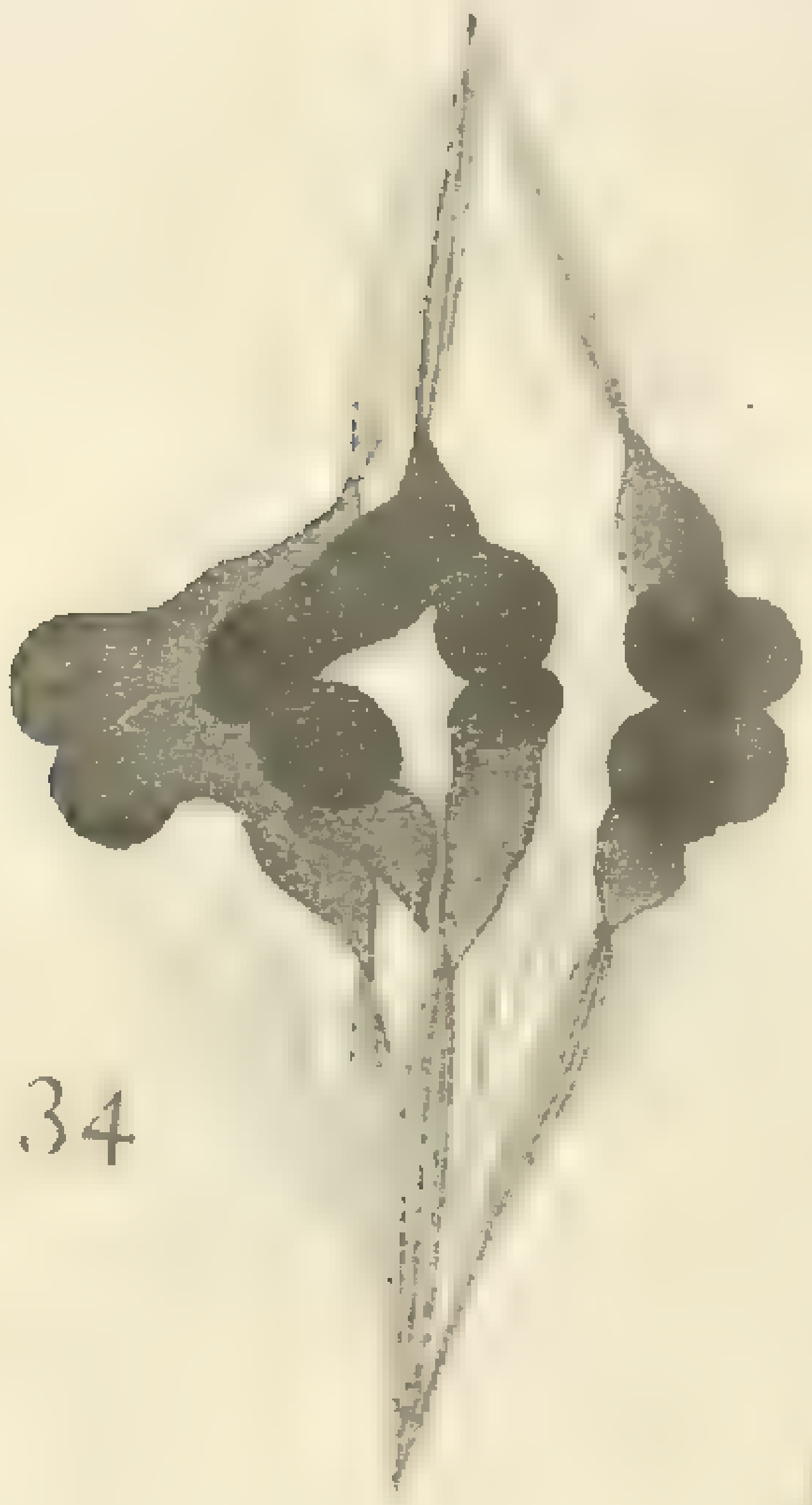
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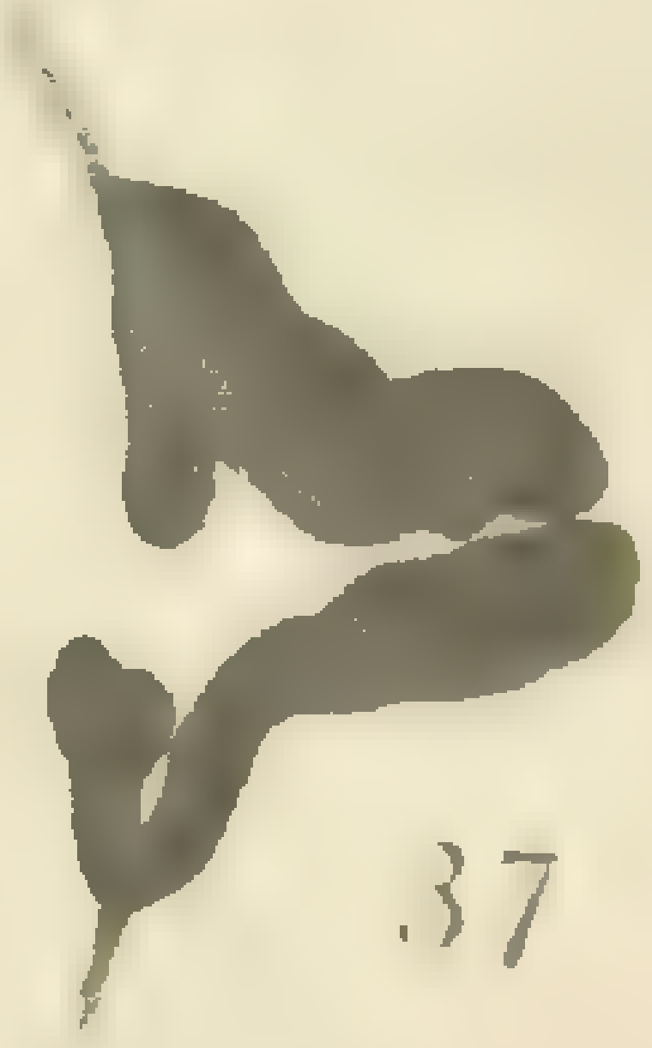
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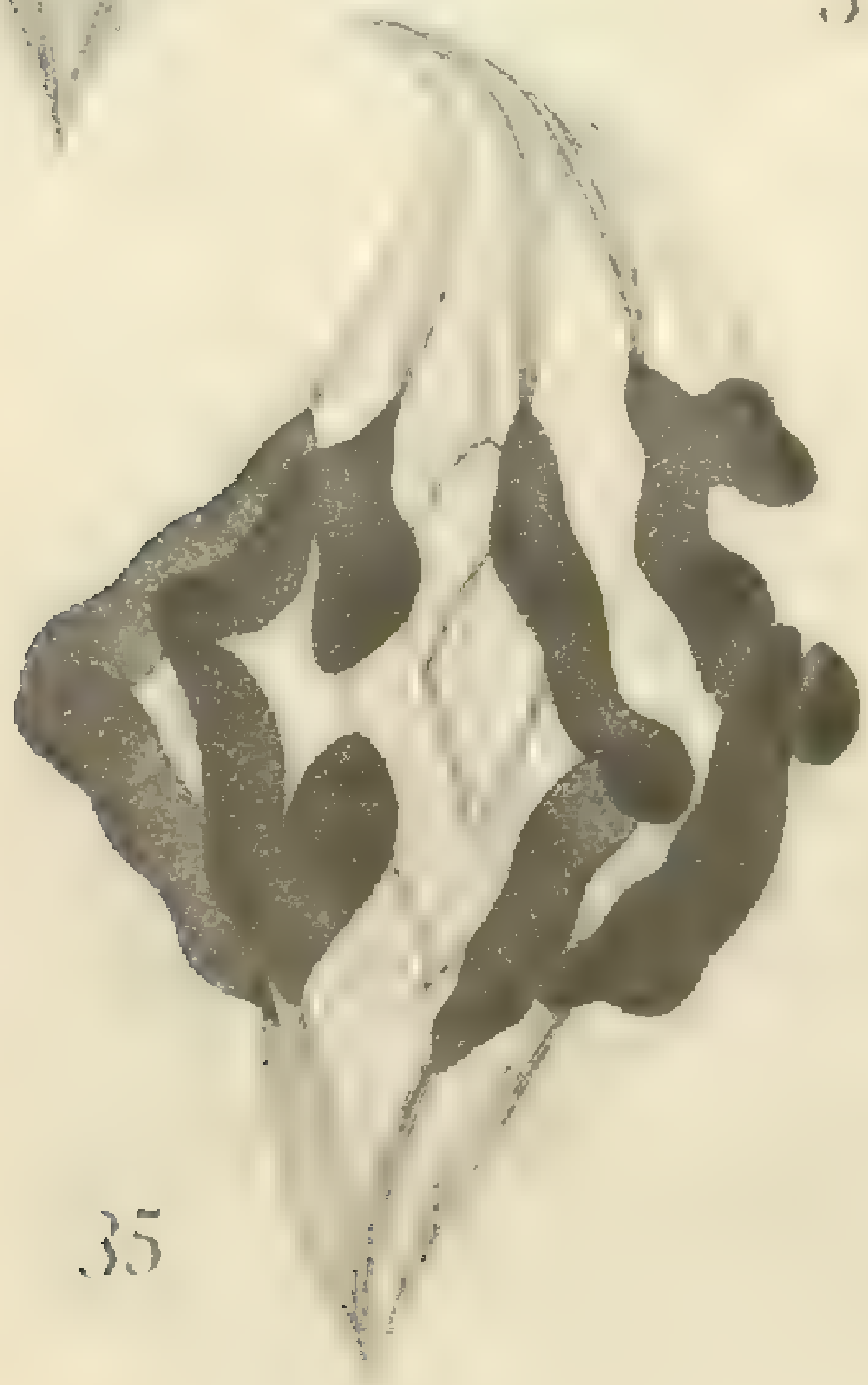
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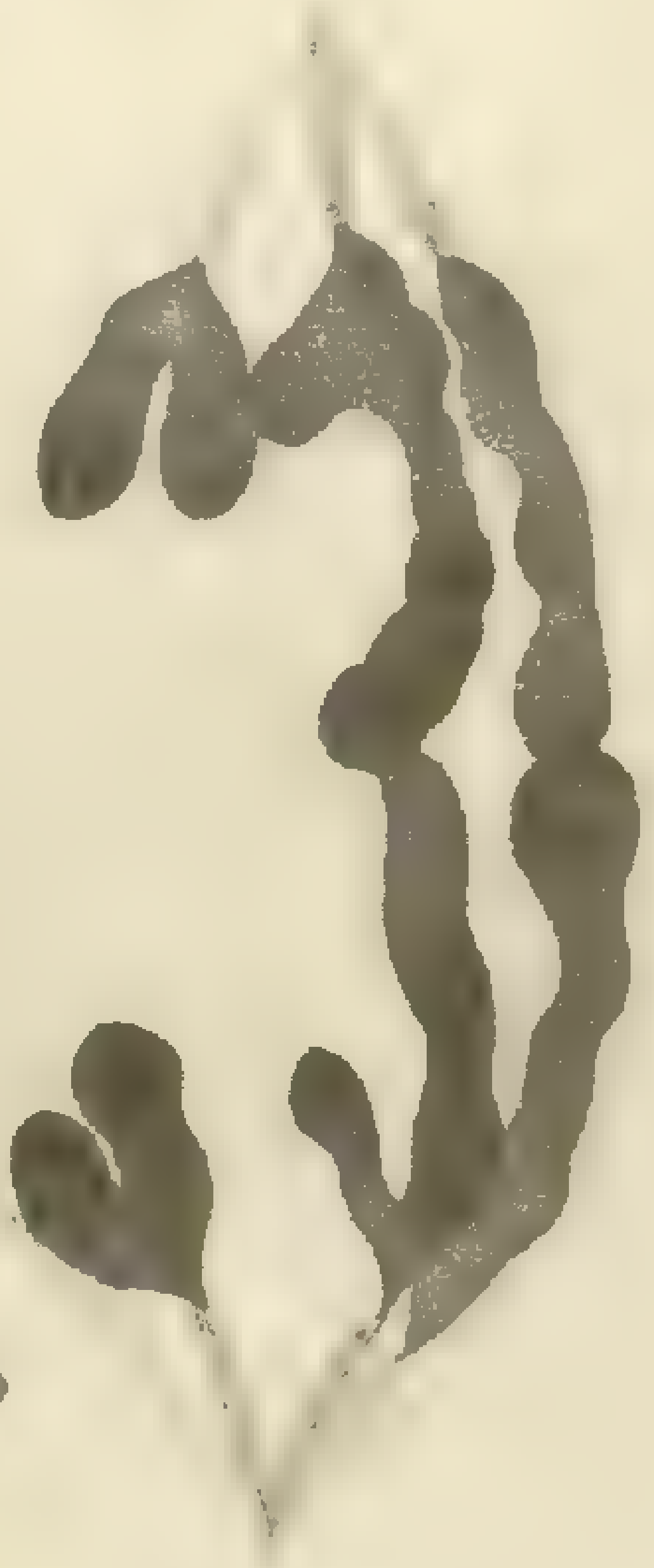
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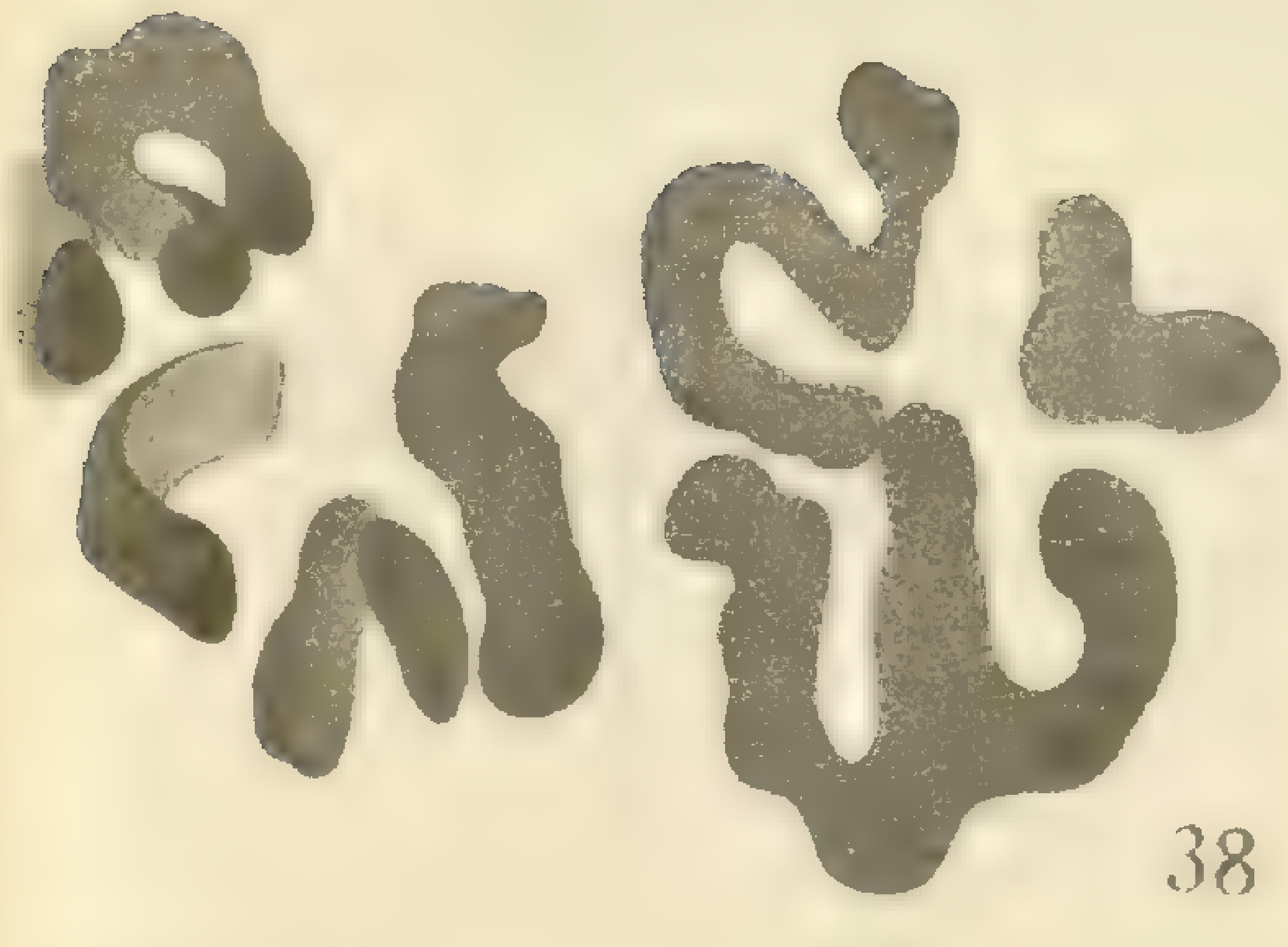
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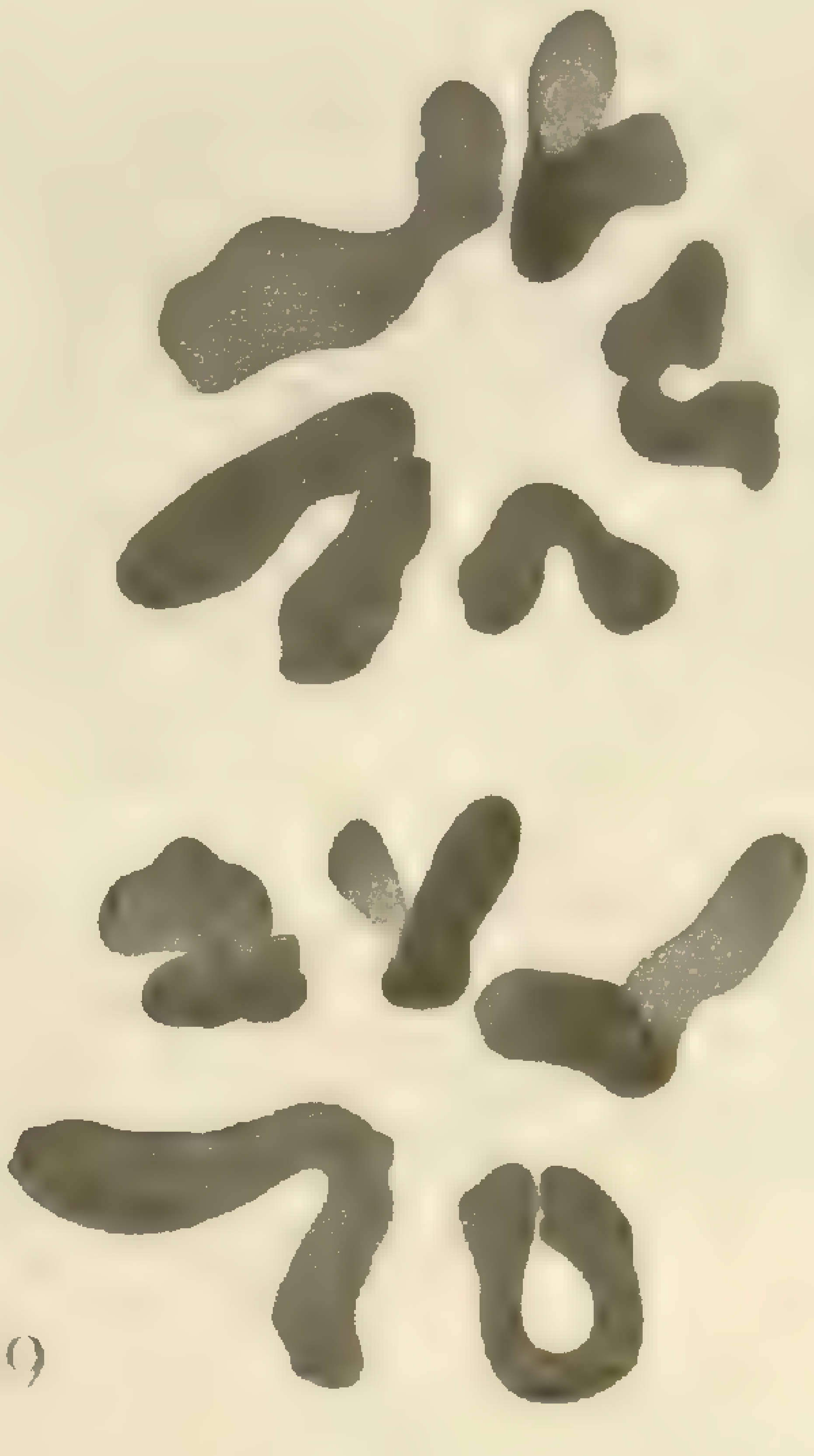
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ATKINSON on REDUCTION of CHROMOSOMES

FLOWERS AND INSECTS. XIX.

CHARLES ROBERTSON.

I. *Comparison of the genera of bees observed in Low Germany and in Illinois, with the number of species of each and their flower visits.*—The results credited to Müller are taken from the *Fertilization of Flowers.* They are based on observations made by Herr Borgstette at Teklenburg, in the north of Westphalia, and by Müller at Lippstadt and in Sauerland, in the central and southern parts of the same region, as well as observations made by him in Thüringia. My results are based on observations made within ten miles of Carlinville. Each species of bee is credited with a visit for each of the species of plants on whose flowers it has been taken.

II. *On the flower visits of oligotropic bees.*—Those bees which visit a wide circle of flowers Loew[1] calls polytropic. On the other hand, the bees which restrict their visits to a few flowers he calls oligotropic. Cases are given by quite a number of authors, but, as far as I can learn, they are cited as mere curiosities; and, as if to keep them more interesting by surrounding them with mystery, the facts which give them significance are omitted. The fact that a species of bee is found on the flowers of one or a few species of plants may only indicate that the bee is rare, or that the entomologist does not know where to look for it. In the economy of the host-bees (those not inquiline) the most important flowers are those from which the female gets the pollen upon which her brood is fed, and we need not trouble ourselves with any cases, or give special names to them, unless it is particularly specified that the female collects the pollen. The more often the female visits a flower without collecting any pollen, the stronger becomes the presumption that there is

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[1] Blumenbesuch von Insekten an Freilandpflanzen. Jahr. Bot. Gartens Berlin 3:—. 1884.

	Westphalia and Thüringia		Macoupin County, Illinois	
	No. species	Visits	No. species	Visits
Sphecodes	17	28	12	74
Prosopis	15	88	7	118
Colletes	4	16	14	96
Halictus	32	440	30	961
Augochlora	—	—	5	232
Agapostemon	—	—	4	132
Andrena	51	219	42	419
Parandrena	—	—	1	13
Nomia	—	—	1	7
Panurginus	—	—	9	52
Perdita	—	—	1	3
Calliopsis	—	—	3	39
Rhophites	2	8	—	—
Rhophitoides	1	2	—	—
Halictoides	1	2	1	4
Panurgus	2	16	—	—
Dasypoda	1	7	—	—
Cilissa	3	16	—	—
Macropis	1	4	1	6
Ceratina	1	3	2	154
Xylocopa	—	—	1	2
Eucera	1	15	—	—
Emphor	—	—	1	4
Melissodes	—	—	18	266
Synhalonia	—	—	4	83
Xenoglossa	—	—	2	5
Entechnia	—	—	1	5
Anthophora	5	32	5	52
Saropoda	1	9	—	—
Melecta	2	3	1	1
Bombomelecta	—	—	1	1
Crocisa	1	1	—	—
Epeolus	1	2	12	113
Nomada	21	85	17	130
Heriades	1	13	3	34
Chelostoma	3	25	—	—
Andronicus	—	1	1	8
Alcidamea	—	—	2	32
Osmia	13	100	10	102
Megachile	9	77	15	225
Chalcodoma	1	1	—	—
Diphysis	1	15	—	—
Anthidium	3	16	1	3
Stelis	3	12	2	7
Coelioxys	6	28	7	66
Neopasites	—	—	2	4
Bombus	13	457	8	456
Psithyrus	4	52	3	12
Apis	1	189	1	157
Totals	205	1,981	251	4,078
Not accounted for		210		
		2,191		

another flower which she does visit for that purpose, and which, therefore, holds a more important relation to her species. Accordingly, I propose to consider those examples in which the female collects pollen of one species, or several species of the same genus or natural family, the relationship of the plants being such as to give significance to the cases. On the other hand, if a bee uses the pollen of only two plants of different families, I assume that it is essentially polytropic, and that the few visits are merely connected with the fact that it is rare or has a short flight. Of course there still remains a strong contrast between the visits of a bee which flies only a month or two and one which flies throughout the season. As a rule, if a bee has a long flight it must be regarded as polytropic, unless the flowers on which it depends have a long blooming time. Of the thirty-nine species of Halictus and the allied Augochlora and Agapostemon, I regard only one as oligotropic, *Halictus nelumbonis*. It has a comparatively short flight, while the blooming seasons of the Nymphæaceæ are long. When a genus of plants has more than one closely allied species, the difference between a monotropic and an oligotropic bee may depend merely upon the accident that only one species occurs in the neighborhood. My observations show that an oligotropic American bee will gather the pollen of a closely related introduced European plant of the same genus.

The relations of the host-bees to the flowers from which they get pollen are quite analogous to the relations of parasites to their hosts, of phytophagous insects to their food plants, or of predaceous insects to the insects upon which they feed or with which they provision their nests. How the bees maintain these relations is much easier to understand, since the flowers are modified in such a way as to facilitate their visits.

Any ecological position is of advantage only to a limited number of individuals. As soon as this *optimum* number is passed, anything which will enable a set of individuals to get along without coming into competition with the dominant form will be of advantage to them, and their preservation will depend upon their adopting this course. A characteristic which would be a disadvantage before the *optimum* is reached, may be an advantage after the *optimum* is passed. Whatever may be the characteristic which enables this set of individuals to hold its own in a new ecological position, I think the principal circumstance which accounts for the adoption of a new mode of life is the pressure of competition. The dominant form retains the original position, the other becomes modified (specialized) in adaptation to the newly acquired position.

In my neighborhood there are thirty-five species of Andrena, which complete their flight from March 17 to July 14. These succeed one another, so that not more than twenty-one would be in competition at the same time, if their habits were the same. Ten begin their flight in March, seventeen in April, seven in May, and one in June.

Of thirty-three species whose habits are pretty well known, nineteen are polytropic and fourteen oligotropic, in the sense in which I use those terms. Four of the oligotropic species get pollen from plants of the same genus, but each of the other ten has its own flower, so there are eleven sets which are absolutely without competition among themselves. I think it is clear that so many species could hardly flourish in the same locality and complete their flight in so short a time, if all were in competition for the pollen of the same flowers.

The average maximum flight of the females is forty-eight days. Now suppose that, on account of the pressure of competition, one of these shifts to a different phenological position. Of the flowers whose pollen is so situated that the bee can readily collect it, only those are available whose pollen is produced in abundance between the time the female is impregnated and the end of the time of flight. To use human terms, the bee must choose between a limited number of flowers, and is in no wise free to regulate its habits according to mere whim.

From the above considerations I do not accept the views of Kerner,[2] although they are the ones adopted by Knuth[3] to explain such cases. Kerner says: "The flowers of the common bryony, *Bryonia dioica*, are not less remarkable. They occur on two kinds of plants, *i. e.*, on one plant are developed only staminate and on the other only pistillate flowers, and since the pollen is not powdery, and therefore not scattered by wind, it must be carried by insects from plant to plant if the ovules are to mature. But the flowers, especially the pistillate ones, are very insignificant, green in color, with faint smell, and they are half hidden under the foliage. Many insects fly past them without noticing them. They are almost exclusively visited by one of the Hymenoptera, viz., *Andrena florea*, and it can find them in the most out-of-the-way places. This can hardly be accounted for except by supposing that the scent of bryony flowers is perceived by these particular bees and not by other insects." He admits these conclusions must be accepted with discretion. *Andrena florea* gets its pollen exclusively from staminate plants of bryony. How much better do we understand the case if we admit that the scent of the flowers is perceived by the bee? The mud-dauber makes its nests of mud and fills them with flower-spiders, which are so near like the color of the flowers which they frequent that they are enabled to capture their prey by lying in wait. Do we explain the case if we say that Pelopœus perceives the scent of mud and Thomisidæ?

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[2] Natural History of Plants **2**: 206. 1894.

[3] Handbuch der Blütenbiologie **1**: 106, 114. 1898.

As for out-of-the-way places, my observations indicate that, as a rule, oligotropic bees nest in the neighborhood where their food plants occur, and that, when the brood emerges next year, it finds the flowers in bloom, and that near by.

As a typical case of an oligotropic bee, *Emphor bombiformis* may be mentioned. Both sexes occur in abundance on flowers of *Hibiscus lasiocarpus*, the female collecting the pollen, the males often spending the night in the flowers. The bees do not occur except when the Hibiscus is in bloom. Within several yards of the Hibiscus I have seen the female making nests in a dry bank, carrying water to soften the earth she was excavating. The bees coming out next year find the Hibiscus in bloom near by. The only visits to other flowers I have seen the bees make were to those in the neighborhood of the Hibiscus. Thus I have seen a single female sucking the nectar of *Cephalanthus occidentalis*, and another that of *Vernonia fasciculata*, as well as a single male sucking nectar of *Ipomœa pandurata*. The outside visits in no way modify the essential relation of the bee to the Hibiscus. For myself, I do not believe in the absolutely exclusive visits of oligotropic bees to their pollen flowers, and I see no reason why they should be expected. If the plants from which a bee gets pollen are common and widely distributed, the proportion of flowers to which it occasionally resorts is much greater than in a case like Hibiscus. Indeed, it strikes me that it is an advantage for the males and unimpregnated females to visit other flowers and not interfere with the females which are collecting pollen. Some bees which stick their pollen with honey get the pollen from nectarless flowers, and so are compelled to visit other flowers for nectar. I have seen *Macropis steironematis*, with pollen balls on her legs, sucking nectar of *Melilotus alba*.

In case of this Macropis and Steironema, Kerner might say the bee perceived or liked yellow flowers, but all of the accessory visits I have seen this bee make were to white flowers, Ceanothus, *Melilotus alba*, Apocynum. In these cases Steironema was in bloom in the neighborhood.

I shall now give the cases of oligotropic bees mentioned by Lubbock,[4] on authority of Müller; by Loew,[5] on authority of Schmiedeknecht; and by Knuth[6] from various sources.

Andrena florea	visits	exclusively	Bryonia dioica.
hattorfiana	"	"	Scabiosa (Knautia) arvensis.
Halictoides	"	"	Campanula spp.
Cilissa melanura	"	"	Lythrum Salicaria.
Macropis labiata	"	"	Lysimachia vulgaris.
Osmia adunca	"	"	Echium.

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[4] British Wild Flowers in Relation to Insects 21. 1875.

[5] Blumenbesuch von Insekten an Freilandpflanzen. Jahrb. Bot. Gartens Berlin 3:274 (72). 1884.

[6] Handbuch der Blütenbiologie 1:114. 1898.

Andrena nasuta	visits	Anchusa officinalis.
cineraria	"	Salix.
lapponica	"	Vaccinium.
cettii	"	Scabiosa (Knautia).
hattorfiana	"	Scabiosa.
florea	"	Bryonia.
alpina	"	Campanula.
curvungula	"	Campanula.
austriaca	"	Umbelliferæ.
lucens	"	Umbelliferæ.

Andrena florea	visits	exclusively	Bryonia dioica.
hattorfiana	"	"	Scabiosa (Knautia) arvensis.
cettii	"	"	Scabiosa (Knautia) arvensis.
nasuta	"	"	Anchusa officinalis.
Bombus gerstaeckeri	"	"	Aconitum lycoctonum.
Cilissa melanura	almost	"	Lythrum Salicaria.
Macropis labiata	"	"	Lysimachia vulgaris.
Osmia adunca	"	"	Echium.
cementaria	"	"	Echium.

Andrena florea, mentioned in the three lists, collects pollen of bryony and has been found on no other flowers.

Andrena hattorfiana: both sexes visit *Scabiosa arvensis*, the female collecting pollen. Müller found a female on *Dianthus carthusianorum* and a male on *Jasione montana*.

Halictoides dentiventris: Müller captured both sexes on *Campanula rotundifolia* and *trachelium*, but not collecting pollen. He says that at St. Petersburg Morawitz found it only on Campanula. In the Alps, Müller observed this species collecting pollen of *Potentilla grandiflora* and *Hypochæris uniflora* and visiting seven other flowers.

Cilissa melanura collects pollen of *Lythrum Salicaria*, the males sucking. Müller saw the female sucking on flowers of *Leontodon hirtus*.

Macropis labiata: males and females visit *Lysimachia vulgaris*, the females collecting pollen. Males suck on *Œnanthe fistulosa*, *Rhamnus frangula*, *Rubus fruticosus*.

Osmia adunca: Müller saw both sexes on *Echium vulgare* and says it feeds its young exclusively on honey and pollen of

Echium, but under *Vicia Cracca* this species is indicated as collecting pollen. In the Berlin garden Loew found it collecting pollen of *Nepeta Mussini*. In the two latter cases there is some error, or the bee is not oligotropic. So of the cases mentioned by Lubbock, only one is exclusive, and two are not even oligotropic.

Andrena cettii: females collect pollen of *Scabiosa arvensis*.

Bombus gerstaeckeri: why Knuth says this species visits exclusively *Aconitum lycoctonum* I do not know, for on page 191 it is distinctly stated that the males and workers visit *A. Napellus*, but nothing is said about pollen-collecting.

Osmia cementaria: males and females suck and collect pollen on *Echium vulgare;* males suck on *Trifolium arvense*.

Of the cases mentioned by Knuth, excepting *Andrena nasuta*, only two are exclusive. *Osmia adunca* and *Bombus gerstaeckeri* are not good cases. In the other cases the females collect the pollen exclusively from the plants set opposite them, and the cases are not essentially modified by occasional visits for nectar to other flowers. I hold that *Macropis labiata* is as good a case as *Andrena florea*.

Of the cases mentioned by Loew, three have been passed upon. I know of nothing against any of them except *Andrena cineraria*. The female collects pollen of Salix, but also of *Taraxacum officinale*, so I should exclude it.

In the observation of the insect visits of flowers correct determinations are very important, for otherwise the records are wrong. One has to be sure that the bee is actually collecting pollen, for often a female bee will suck nectar from a flower when her scopæ are full of pollen from another species. On the other hand, there is danger of assuming that a bee is oligotropic from too few observations.

A neighborhood where the flora and insect fauna are in their normal condition is more favorable for correct observations on oligotropic bees; for, when the flowers upon which a bee depends become extinct or rare, the bee may disappear or be forced to resort to flowers which originally it did not visit. In most cases the former is more likely to happen.

A bee may be regarded as oligotropic: (1) When the female collects the pollen of the plants in question and is not known to collect pollen of any other plants. (2) When the bee does not occur except during the blooming season of the flowers. If the female is shown to occur after the flowers have quit blooming, the case is very doubtful. (3) When the bee is frequent upon the flowers, and more or less rare upon other flowers, at any rate except in the neighborhood of the food flowers. The case is also doubtful if it is shown that the distribution of the bee extends greatly beyond the plants upon which it is supposed to depend.

In the more satisfactory cases, if any one should say that he had observed the bee collecting pollen from a quite unrelated flower, I would not accept the determination, or, if that were beyond question, the opinion that the pollen came from the flower on which the bee was taken.

Below I give a list of bees which I regard as oligotropic in the above sense. When I have observed the female collecting pollen from more than one species of a genus, I give the genus; when from more than one genus, I give the family. The details will be given elsewhere.

In Prosopis the females are destitute of pollinigerous apparatus, their nests being provisioned with a paste of honey and pollen. I know of no way to distinguish the flowers which the females visit for this purpose from those which they visit in only an incidental way, so I assume that a species of this genus is oligotropic only so long as it is found exclusively on flowers of one species or group. This may be assumed for either sex so long as the condition holds, as in case of *P. illinoensis*, of which I do not know the females. *P. nelumbonis* has always seemed to me to be the best case of an oligotropic Prosopis.

I have never believed that our species of Epeolus were cuckoos of Colletes, because there are more common species of the former than of the latter genus, and their phenological positions do not show the same correlations which exist between Andrena and Nomada, Megachile and Coelioxys. Besides, the maximum of Epeolus does not approximate that of any other

Bee	Plants visited by females for pollen	Number of species	Flowers of same genus visited for nectar	Flowers of same family visited for nectar	Other flowers visited for nectar	Total flowers visited for nectar
Colletes aestivalis	Heuchera hispida	1	—	—	4	4
latitarsis	Physalis	3	—	—	6	6
willistonii	Physalis lanceolata	1	—	—	3	3
americanus	Compositæ	8	—	2	3	5
armatus	"	4	—	1	1	2
compactus	"	8	—	2	—	2
eulophi	"	3	—	3	11	14
Andrena arabis	Arabis lævigata	1	—	—	—	—
erigeniæ	Claytonia Virginica	1	—	—	2	2
geranii	Hydrophyllum appendic'um	1	—	—	2	2
g. maculati	Geranium maculatum	1	—	—	1	1
polemonii	Polemonium reprans	1	—	—	2	2
spiræana	Spiræa Aruncus	1	—	—	3	3
violæ	Viola cucullata	1	2	—	3	5
erythrogastra	Salix	4	1	—	7	8
illinoensis	"	4	1	—	8	9
mariæ	"	4	—	—	6	6
salicis	"	4	—	—	2	2
nasonii	Umbelliferæ	3	—	—	1	1
ziziae	"	5	—	—	—	—
rudbeckiæ	Rudbeckia hirta	1	—	1	—	1
aliciæ	Compositæ	5	—	2	—	2
asteris	"	3	—	—	1	1
helianthi	"	3	—	2	—	2
nubecula	"	4	—	1	—	1
pulchella	"	6	—	2	—	2
solidaginis	"	6	—	1	1	2
Parandrena andrenoides	Salix	3	1	—	9	10
Macropis steironematis	Steironema	3	—	—	3	3
Halictus nelumbonis	Nymphæaceæ	3	—	—	—	—
Megachile exilis	Campanula Americana	1	—	—	6	6
pugnata	Compositæ	4	—	1	3	4
Panurginus labrosus	Rudbeckia triloba	1	—	2	—	2
albitarsis	Compositæ	2	—	4	—	4
asteris	"	4	—	—	—	—
compositarum	"	5	—	3	1	4
labrosiformis	"	7	—	3	—	3
rudbeckiæ	"	4	—	—	—	—
rugosus	"	4	—	2	—	2
solidaginis	"	2	—	4	—	4
Xenoglossa pruinosa	Cucurbita Pepo (cult.)	1	—	3	—	3
Emphor bombiformis	Hibiscus lasiocarpus	1	—	—	3	3
Anthophora walshii	Cassia Chamæcrista	1	—	1	4	5
Perdita octomaculata	Compositæ	3	—	—	—	—
Halictoides marginatus	Helianthus	3	—	1	—	1

Bee	Plants visited by females for pollen	Number of species	Flowers of same genus visited for nectar	Flowers of same family visited for nectar	Other flowers visited for nectar	Total flowers visited for nectar
Mellisodes desponsa	Cnicus	2	1	—	1	2
illinoensis	Lepachys pinnata	1	—	—	1	1
agilis	Compositæ	6	—	12	10	22
americana	"	9	—	2	1	3
coloradensis	"	7	—	6	1	7
pennsylvanica	"	6	—	9	3	12
simillima	"	6	—	12	3	15

genus of bees on which it might be supposed to be inquiline. Then they are more abundant than would be expected of inquiline bees. Mr. Ashmead's observations confirmed my views, and I have never doubted their correctness since I first read an account of them. In *Psyche*, for March 1894, p. 41, he states that he found *E. donatus* making nests in the ground and provisioning them with a honey-paste. Epeolus thus comes under the same category as Prosopis and is treated the same way in the table.

The cuckoo bees of the genus Nomada hold no particular relations to flowers except through their hosts. However, they show considerable differences. *N. vincta*, which is common on Helianthus and was taken once on Coreopsis, is, I think, an inquiline of *Andrena helianthi*, both bees occurring at the same time, in the same neighborhood, and on the same flowers.

Bee	At least females visit exclusively	No. spp.	Other fls. visited by male
Prosopis nelumbonis	Nymphæaceæ	2	—
thaspii	Thaspium aureum trifoliatum	1	—
illinoensis	Umbelliferæ	5	—
Epeolus helianthi	Helianthus grosse-serratus	1	1
compactus	Compositæ	4	—
cressonii	Compositæ	13	3
pectoralis	Compositæ	2	—
pusillus	Compositæ	4	—
Nomada vincta	Compositæ	3	—

III. *Competition of flowers for the visits of bees.*— It is a question to what extent groups of plants adapted to certain kinds of bees should be regarded as in competition and to what extent they should be regarded as mutually helpful. We will suppose a case in which a plant whose flowers may be visited by bees is introduced into a region where all visitors must be acquired. If the region contains no flowers, there will be no bees to acquire. On the other hand, it seems to me that the more nearly the flora retains its original characteristics the more bees there will be and the more chances there will be of the new flower acquiring bees as visitors. My view is that a patch of plants adapted to bees of certain kinds will be more abundantly visited, if it is surrounded by plants depending on bees of the same kinds, than if the neighboring grounds are unoccupied. There will be more of these bees in the neighborhood. In the table there are fifty-two species which get pollen from particular plants. As far as the data are correct, we take it for granted that the presence and abundance of these bees in a given locality depend on the presence and abundance of the flowers from which they get their pollen. One object in making the table is to show that the plants growing in the neighborhood of plants visited by oligotropic bees gain a certain number of bee visits. The table shows that these plants gain 204 visits in this way. It is expected, however, that some of the visits enumerated in the second and third columns will have to be transferred to the first. Excluding these columns, the neighboring unrelated plants gain 116 visits from the proximity of the food-plants of oligotropic bees. It is not likely that a plant suited to the visits of different kinds of bees will show the normal circle of visitors unless it holds its normal position in the original flora.

IV. *On the influence of bees in the modification of flowers.*— The facts indicate that the first entomophilous flowers were visited for nectar. Anemophilous flowers offer such a poor foothold for insects that they are very seldom visited by them, and the pollen, although no doubt palatable to many insects, is so light and dry that it is apt to be blown away as soon as it is liberated from the anthers. The first step in the development of entomophilous flowers was the secretion of nectar somewhere about the stamens and pistils, correlated with the modification of the flower so as to afford convenient resting places for insects, and the pollen becoming more adhesive, so that it would remain on the anthers after dehiscense and become attached finally to the bodies of the guests. The object of insect visits being the nectar, modifications favoring cross-pollination resulted in the various forms of diclinism and dichogamy. The perfection of nectar-bearing flowers naturally reached a high grade in the less specialized groups of plants, as, for example, the orchids, and was most frequently associated with the less specialized anthophilous insects.

Along with the development of convenient landing places and sticky pollen, there has no doubt been an increasing number of insects which resorted to flowers for pollen. Finally, the most highly specialized of anthophilous insects, the Hymenoptera, gave rise to a still more highly specialized group of insects which adopted the habit of provisioning their nests with nectar and pollen. Along with the acquisition of this habit the bees developed a coat of feathery hairs to which the pollen might cling, these hairs on certain parts of their bodies, as the hind legs and ventral surface of the abdomen, being greatly modified to form special pollen-carrying apparatus called scopæ. Thus the pollen became absolutely essential in the economy of the most highly specialized anthophilous insects. To the flowers, on the other hand, the bees became the most important visitors, because they had to resort to flowers more frequently than other insects, and because they were provided with a coat specially fitted to retain the pollen, and at the same time exerted themselves to get the coat as full of pollen as possible.

That the development of entomophilous flowers with sticky pollen preceded the development of the bees is indicated by the fact that the less specialized bees only collect adhesive pollen. The most highly specialized bees, however, have acquired the habit of sticking the pollen with honey, and so can use that of anemophilous plants.

Those flowers, however, which, through their nectar and correlated modifications, were the best fitted to use the services of ordinary insects for cross-pollination, were the least fitted to utilize the insects which were the highest product of anthophilous development. Strange as it may seem, the characters which hindered them from availing themselves of these services were the very characters which are considered the highest adaptations for cross-pollination, viz., diclinism, dichogamy, and large size. On the other hand, the forms which have enabled flowers most readily to avail themselves of the services of bees are the very characters which have been interpreted as adaptations for self-pollination and geitonogamy, viz., small size, homogamy, and the aggregation of dichogamous and other flowers in close clusters.

If an insect in search of nectar visits a diœcious or other diclinous plant, it is not hard to understand how it is likely to visit both staminate and pistillate flowers and readily effect cross-pollination. It is not so certain that a female bee in search of pollen will visit the pistillate flowers in anything like the same degree. Indeed my observations lead me to believe that they do not. I have seen hive-bees in great numbers collecting the pollen of *Salix humilis* and paying no attention to the pistillate flowers. They fairly monopolized the staminate flowers, while the pistillate flowers were visited by an entirely different set of insects. In the table there are six species of bees which get their pollen exclusively from diœcious species, Salix and *Spiræa Aruncus*. Of the plants furnishing pollen to oligotropic bees, these are the least able to utilize these bees on account of their diœcism.

Dichogamous flowers are at somewhat of a disadvantage in utilizing pollen-collecting bees from the fact that the bees are more apt to pay attention to the flowers which are discharging pollen and neglect those in the other stage. In *Impatiens fulva* and *I. pallida* I have observed that *Megachile brevis* collects the pollen from flowers in the first stage and avoids those with receptive stigmas, because she instantly perceives that the anthers are gone. *Apis mellifica* and *Bombus virginicus* do the same when collecting the pollen of *I. fulva*. In *Campanula Americana*, which is also proterandrous, the oligotropic *Megachile exilis* cleans the pollen from the style-brushes before the stigma opens, and avoids the old flowers. In *Lobelia syphilitica* I have seen little bees collecting the pollen which was pushed out of the anther tube before the stigma appeared. In the proterandrous *Monarda Bradburiana* I have seen small bees collecting pollen directly from the anthers, avoiding the old flowers. The strongly dichogamous flowers mentioned in the table are not so well adapted to utilize their special visitors as are the homogamous ones, such as Viola, Psoralea, Hibiscus, Cassia, because in the latter the bees cannot collect the pollen without touching the stigmas.

Some dichogamous flowers may make effective use of the pollen-collecting bees, as in the case of *Nymphæa reniformis*, which, in my opinion, is proterogynous and without nectar. By a sudden bending of the filaments, bees alighting on the anthers are let down into the stigmatic basin before they discover that the pollen is not being discharged. Of course, in other dichogamous flowers the bees may visit the flowers in the pistillate stage before they discover that the pollen is gone, or for nectar, but my observations have convinced me that this is not the rule, for if they do not know exactly what they are doing and how to do it, they act just like it. On their pollen-collecting expeditions they do not make many mistakes or waste much time.

Even some homogamous flowers are so large that the smaller bees may collect their pollen without touching the stigmas. This may not matter so much if the flowers are visited by large bees, which are more effective. But the smaller flower may, in many cases, utilize the large bees as well and the smaller ones better. So I think the influence of the pollen-collecting bees is in favor of the smaller homogamous flowers.

Under the influence of the nectar-sucking, less specialized, anthophilous insects the highest development is found in diclinous, dichogamous, and hercogamous flowers with highly specialized nectaries and precise localization of pollen contact. In the less specialized plants, this kind of adaptation early reached the highest degree of perfection in the case of the orchids. But, as far as I know, no orchid holds an important relation in the economy of any bee.

Under the influence of the female bees, the most highly specialized of anthophilous insects, the highest development is found in homogamous flowers without nectar, such as Desmodium and Cassia.

Since bees have entered the field, many flowers seem to have been at a disadvantage in gaining their services, because the stamens were so few that they could not offer pollen in paying quantities. And in many cases the stamens were covered by galeæ and carinæ, so that, to collect the pollen, the bee would have to spend much time going to the bottom of every flower. This difficulty was obviated by lengthening the stamens, reducing the size of the flowers, and crowding the flowers so that the bees could run over or around the inflorescences and sweep up immense quantities of pollen. Inflorescences of this kind are found in Cornus, Hydrangea and Viburnum.

Here we find an explanation of the fact that certain Leguminosæ and Labiatæ have abandoned their galeæ and carinæ, exposing their stamens, and contracting their infloresences into head-like or flat-topped clusters, as in Amorpha, Petalostemon, Lophanthus, Mentha, Blephilia, and Pycnanthemum. Contrary to Müller, I think Delpino is right in regarding Mentha as one of the most highly specialized of the Labiatæ, and I incline to the same opinion regarding the above genera of Leguminosæ. These cases are obscured by the fact that the arrangements permit the visits of a lot of less specialized insects. Nevertheless I think the bees have determined the result.

In the case of Lobelia I have mentioned that small bees collect the pollen pushed out of the tube before the stigma appears. In the Compositæ we find plants perhaps best adapted to attract and utilize the pollen-collecting bees, and the table shows that they have among their visitors more oligotropic bees than any other group of native plants, and that, too, in spite of their dichogamy. If the flowers were greatly scattered, they no doubt would not attract so many bees, and the bees could carry off the pollen and not render any service by visiting the flowers after the stigmas appeared. But, as a result of the reduction of the flowers in size and the crowding of them in heads, we find a circle of flowers, each one of which ejects the contents of five anthers in a convenient mass. Just without is a circle of flowers with protruding stigmas. Bees sweep over the disk, filling their pollen-scopæ with the greatest facility, at the same time effectually pollinating the neighboring stigmas.

As the homogamous flowers have largely been given over as adaptations to autogamy, so the crowded inflorescences have been given over as adaptations to geitonogamy. As a category I do not accept Kerner's *geitonogamy*. Kerner regards most of the crowded inflorescences as adaptations for geitonogamy, and founds a special category for their reception. This is accepted by Knuth and is incorporated in his recent Handbuch.[7] I do not believe in any adaptations for geitonogamy. I do not deny that it occurs, and under pseudo-ecological conditions may be advantageous, but it is only the name of an accident and does not account for any floral adaptations. Kerner does not make a distinction between a structure, or habit, which has a certain effect, and one which may be conceived to be developed for a certain purpose, or selected on a certain condition. He even speaks of a "contrivance for securing hybridization." Under "contrivances whereby the pollen is protected against wet" he says: "In *Podophyllum peltatum* the pollen is sheltered by the bell-shaped flower, but in addition to this the peltate foliage-leaves are also spread out over the flowers and act as umbrellas." Under the category of protection by isolation in water he mentions a number of ordinary water plants and says: "Flies and beetles which come through the air for honey and pollen are welcome visitors, promoting, as they do, a crossing of the pollen; snails, centipedes, etc., are, on the other hand, kept back by the water." He gives no evidence that this protection has anything to do with the fact that the plants have acquired an aquatic location. He uses trivial and accidental effects as a basis for interpretation of all kinds of ecological phenomena.

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[7] Handbuch der Blütenbiologie 1: 51.

While it is true that adaptations for cross-pollination are more apparent in the less specialized plants depending on the less specialized anthophilous insects, it does not follow that the adaptations of the highest plants in relation to the highest insects, though more obscure, are to be interpreted as arrangements for autogamy and geitonogamy.

V. *On the supposed pollen-carrying apparatus of flies and birds.*—In regard to the plumose aristæ of such genera of Syrphidæ as Volucella and Sericomyia, Loew[8] observes that the structure appears of no use to the flies, but is of importance in the transfer of pollen. And he regards them, as well as the hairy coat on the lower part of the face, as an adaptation for carrying pollen. In the same connection he mentions the hairy eyes of certain species, though he does not go so far as to consider this as an adaptation for the same purpose.

In the *Entomological News* **4**: 323. 1895, under the title *Insects as pollenizers*, Mr. J. B. Smith mentions that some Diptera have compound hairs, similar to those found in the Apidæ. The author does not say exactly what he does mean, but I have always regarded the note as implying the view that these hairs were so modified for carrying pollen.

In the *American Naturalist* **28**: 680–681. 1874, Mr. J. L. Hancock speaks of certain "repositories" on the head of the ruby-throated humming bird, and throughout his paper seems to imply that the feathers, etc., are specially modified for carrying pollen. As Mr. Darwin says, proof of the existence of such adaptations would be fatal to the theory of natural selection. I have always regarded these statements as mere teleological curiosities, but in his *Handbuch* Knuth has adopted Loew's views.

[8] Jahr. Bot. Gartens Berlin **6**: 114. 1886.

which has the effect of giving them some standing among the fundamental principles of flower-and-insect ecology.

The existence of branched hairs in the bees may properly be interpreted as an adaptation for carrying pollen, because the bees use them for that purpose, and the importance of the hairs is evident, in view of the economy of the insects. They cannot in any way be interpreted as existing for the benefit of the flowers. It could be of no advantage to flies and birds to carry pollen, since they make no use of it. However, it might be claimed that these guests derived an indirect benefit from the pollination of their favorite plants. But their relations to flowers are not close enough to make their existence depend upon the pollination and preservation of any particular species.

An examination of the inquiline bees will lead to the conclusion that the several genera are not related to one another but have arisen independently from different groups of host bees. It will also lead to the conclusion that they have all lost their hairy coats, or tend to do so, as in Psithyrus. To my mind the fact that these bees began to lose their coats as they abandoned their pollen-collecting habits, involves a clear refutation of the claims that any structures on flies and birds were developed for the purpose of carrying pollen.

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THE ORIGIN OF THE LEAFY SPOROPHYTE.

JOHN M. COULTER.

ATTENTION has been called afresh to this exceedingly interesting and obscure problem by the discussion of alternation of generations by Professor Bower in his recent presidential address,[1] and in papers of Dr. Klebs,[2] and Dr. Lang.[3] The remarks of Professor Bower are largely in defense of his theory of the antithetic origin of the sporophyte, which had been attacked by Dr. Scott in his presidential address of two years before in restating Pringsheim's theory of homologous alternation. In defending his position, Professor Bower discusses arguments derived from the behavior of algæ and certain fungi, from bryophytes, and from apogamy and apospory. He claims that those algæ and phycomycetes which show subdivision of the zygote into spores appear to offer the "key to the enigma" of the origin of the sporophyte, but he makes no further claim for these "fruit bodies" than that they suggest the way in which the sporophyte may have arisen, his view not at all involving the idea that these "fruit bodies" and the sporophyte are homogenetic. He calls attention to the fact that knowledge of cytological phenomena among algæ and fungi is far too meager, especially in connection with the divisions of the zygote referred to. If reduction is found to occur in connection with the zygote divisions, in such forms as Œdogonium and Coleochæte, there would be a reasonable foundation for the belief that the "fruit bodies" are the correlatives of a sporophyte, the beginning of a neutral generation.

In reference to the bryophytes, Professor Bower sees in them a good illustration of the origin of the sporophyte by "a progressive antithetic alternation." He calls attention to the remarkable constancy of alternation in this group, apogamy and apospory being singularly absent. This undeviating alternation he suggests may be accounted for by the dependence of the sporophyte, which is in an "equable physiological condition." As a contrast to this, the independence of the pteridophyte sporophyte, and its exposure to varied conditions, may have caused more freely unusual developments. The primitive pteridophyte, however, was probably in a dependent condition, as the embryos of modern pteridophytes are.

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[1] Nature, Nov. 17, Nov. 24, Dec. 1. 1898.

[2] Annals of Botany **12**: 570–583. 1898.

[3] Annals of Botany **12**: 583–592. 1898.

Apogamy and apospory Professor Bower would regard as "abnormalities," calling attention to the fact that these phenomena have their "headquarters" in the leptosporangiate ferns, a peculiarly specialized phylum with many other abnormalities. Even when apogamy occurs the archegonia are first produced, indicating the "first intention" of the plant; and in both apogamy and apospory the growths may be very anomalous.

In this connection, Professor Bower makes a very interesting suggestion, based upon the experiments of Dr. Lang and others. He observes that apogamy is induced by prevention of contact with fluid water ("rendering fertilization impossible"), exposure to direct sunlight, and possibly to certain temperature conditions. All this leads to a "plethoric" state, which he thinks may be a necessary condition preceding apogamy, as opposed to deficient nutrition, which precedes apospory, the latter being "a physiological refuge for the destitute plant." He suggests that nuclear changes may accompany these conditions, plethora doubling the chromosomes, and hence inducing the development of a sporophyte; and deficient nutrition reducing the chromosomes, thus making a gametophyte possible. Of course it remains to be proved that nuclear instability, coming to be well recognized, is connected with disturbed nutrition, and also whether a smaller or larger number of chromosomes necessarily determine a gametophyte or a sporophyte.

On the whole, therefore, Professor Bower still maintains that the sporophyte is the result of the gradual elaboration of the zygote, "a fresh phase having thus been gradually interpolated," in other words, that its origin is antithetic. It would seem that in his opinion the sporophyte has probably appeared in just one way. This does not mean that all sporophyte plants are homogenetic, but that all have had an origin similar to that of the sporogonium of bryophytes. Professor Bower acknowledges that the present tendency is toward a comprehensive polyphyletic view as regards alternation, stating that "when difficulties arise refuge is taken in the plausible suggestion of distinct lines of descent."

Dr. Lang's paper is rather a presentation of current views than an expression of opinion in reference to any of them. He recognizes the fact that the regularity of the zygote product in such forms as Œdogonium and Coleochæte represents a life history decidedly different from the homologous alternation of sexual and asexual plants in most thallophytes. From one point of view this zygote product is merely a reduced asexual individual; from another point of view it is not a reduced asexual individual, but a special adaptation to multiply the product of fertilization. The former is the theory of homologous origin, the latter the theory of antithetic origin. Certainly the facts of morphology do not decide which theory is correct. Dr. Lang calls attention to the fact that in considering alternation the possible polyphyletic origin of the archegoniates must be kept in mind, as the pteridophytes may represent an entirely distinct line from the bryophytes, as suggested by Goebel. In spite of Professor Bower's disposition of apogamy as an argument, Dr. Lang thinks that experiments with this phenomenon indicate so clearly that the gametophyte may assume characters of the sporophyte under suitable conditions, almost a complete series of transitions between gametophyte and sporophyte having been observed, that such a general property of the fern gametophyte cannot be disregarded in the discussion, even though the phenomenon may be called teratological. He thinks that apogamy suggests the homology of the gametophyte and sporophyte, and may suggest how pteridophytes could have been derived from algæ forms, and how alternation in ferns might have arisen if it did not come antithetically.

The paper of Dr. Klebs deals with the subject of alternation of generations in thallophytes, and therefore concerns this present discussion but indirectly. His experiments among the lower forms, as is well known, have proved that there is no such rigidity in life histories as was once supposed. As a consequence, he does not consider that there is any such thing even as a regular homologous alternation of sexual and asexual phases. He thinks that experiments may prove that the so-called "fruit bodies" of such forms as Œdogonium and Coleochæte may turn out to be the result of certain conditions, rather than an inevitable part of the life history. He seems to consider that the origin of pteridophytes probably has nothing to do with that of bryophytes, and that there is at present no clue whatsoever as to the origin of the former. Such a peculiar structure in common as the archegonium he suggests may be a purely parallel development, without necessarily indicating any phylogenetic connection.

It will be seen from the above papers that, while the origin of the sporogonium of bryophytes seems to be suggested, the origin of the leafy sporophyte is too obscure to justify any definite claim. According to Bower it is most probable that it is developed from such a sporogonium structure as is displayed by the bryophytes today; according to Lang and Klebs there is a possibility that it may have had an entirely independent origin, and may never have been in the sporogonium condition.

It is recognized that there are peculiar difficulties in the discussion of such a subject. Although the morphology of the existing representatives of the various groups is fairly well known, there are two enormous gaps in our knowledge which make a definite conclusion impossible. One of these gaps is the ancient history of the bryophyte and pteridophyte lines. For instance, it is certain that the pteridophytes were well represented in the palæozoic, probably even in its earliest periods. This represents such a tremendous stretch of time that almost any change, however extensive, may have been possible in any given form. It is

Mo. Bot. Garden,
1900.

not through lack of time, therefore, that one would suggest that it is unlikely for a leafy sporophyte to have been developed from a sporogonium. From the fact that our earliest evidences of the pteridophytes show them to have been about as highly differentiated as they are now, it is evident that the evolution of the line reaches very far back. It is probably hopeless to expect that this gap in our knowledge will be filled.

The other gap is in reference to cytological details. The whole subject of alternation of generations seems to be so bound up with nuclear changes that a knowledge of these in the thallophytes becomes a very great desideratum. This gap in our knowledge is likely to be filled up rapidly. It may be that we have been too rigid in our use of the number relations of chromosomes as distinguishing gametophytes from sporophytes. Be this as it may, there is enough in the testimony associating the doubling and the reduction of chromosomes with the sporophyte and gametophyte stages to justify such use. It would seem that an investigation into the nuclear changes which occur in the "fruit bodies" of such forms as Œdogonium and Coleochæte would go far toward settling the antithetic origin of such a structure at least as the sporogonium of bryophytes.

It is not my purpose in this paper to traverse ground which has been gone over so recently and so ably, but merely to discuss certain facts and possibilities in connection with the leafy sporophyte that may be suggestive. In discussing the origin of such a structure as the leafy sporophyte where there is no possible direct evidence, and where every view must be hypothetical, it seems necessary to consider all possible alternatives. The chief service which these various alternatives render is to coordinate the facts and to suggest lines of research.

No structure among plants seems to have left so little trace of its origin as the leafy sporophyte of pteridophytes and spermatophytes. The evolution of the leafless sporophyte of bryophytes seems traceable from an oospore which directly organizes a group of sporogenous cells. Sterilization of the peripheral cells would result in a simple spore case like that of

Riccia, while further encroachment upon the sporogenous tissue, with more or less differentiation of the sterile tissue, would account for the series of sporogonia displayed by bryophytes. Whether the origin of this structure is to be regarded as homologous or antithetic is not pertinent to the present discussion, but it seems reasonable to see in it an entirely new structure developed by the oospore, and in no way homogenetic or even homologous with the gametophyte. It has been noted that the argument drawn from apogamy in favor of homologous origin finds little or no application among bryophytes, for the origin of the sporogonium seems to be as fixed as the origin of any plant structure can be.

It has been common to regard the distinct sporophyte as having been established once for all by the bryophytes, and the sporophytes of the higher groups to have been derived from those of the bryophytes. In searching for the origin of the leafy sporophyte, therefore, attention has been focused upon the sporogonia of bryophytes, and the Anthoceros forms have been selected as most nearly representing the ancestral condition.

The doctrine that any plant structure, however important, can have but one phylogeny, is hardly tenable at present. That heterospory has appeared independently in several lines has become evident; and that it has resulted more than once in seed formation is hardly less evident. The conditions which determined these modifications must have been common enough to have established similar results more than once. Why the sporophyte may not fall in the same category is not clear. Professor Bower's statement that the polyphyletic origin of a structure is an easy escape from difficulties suggests caution, but does not close the door to the fact that nature may have found the same easy way out of difficulties.

In contrasting the sporophytes of bryophytes and pteridophytes, they seem to have nothing in common except that they are usually derived from the oospore and represent an asexual generation. These facts are important, but so are the numerous other facts in which they differ sharply. There are also asexual generations derived from oospores among thallophytes, but regular alternation of sexual and asexual generations is not definitely established. When alternation becomes definite the sporophyte is a recognizable structure, but that this structure must have been established just once or in just one way is far from necessary.

It may be well to contrast the leafless and leafy sporophytes. In the former case the structure is never independent of the gametophyte, develops no lateral members, has nothing comparable to sporangia, and its whole tendency is to render complex the spore-producing region. In the latter case the sporophyte is dependent upon the gametophyte only in its embryonic stage, develops prominent lateral members, has distinct simple sporangia, and its whole tendency is to render complex the sterile or nutritive tissues. As one traces the evolution of the bryophyte sporogonia they give evidence of increasing complexity and hence rigidity, and little promise of originating such a diverse tendency as that shown by the sporophyte of pteridophytes. The mosses are conceded to be a highly specialized, and hence non-productive line, the legitimate outcome of the whole bryophyte tendency. Why the liverwort lines may not also be regarded as highly specialized and hence non-productive does not seem clear. It is true that the Anthoceros forms show a sporophyte tendency unlike the others, and that if such a sporophyte should become independent and put out leaves, and if the continuously developing spore region should be restricted and broken up into simple sporangia which should associate themselves with the leaves, we might have something like the existing leafy sporophytes. But there is no evidence that these things ever happened. On the contrary, the sporophyte of Anthoceros would seem to be as hopelessly specialized as that of other lines. It is true that all the things referred to above may have happened, and Anthoceros may be the nearest living suggestion of the archetypal pteridophyte, but the case is not so clear that our eyes should be shut to other possibilities.

If the bryophyte sporogonium is responsible for the leafy sporophyte, then it is evident, as Bower has shown, that the leaves of the latter are the result of progressive sterilization, and are secondary structures of the sporophyte. But if some other origin of the leafy sporophyte is possible, the leaves may not have arisen as secondary structures.

It may be well to trace briefly the origin of gametophyte leaves, as exhibited by the mosses, since the sequence of events seems fairly clear, and may prove suggestive. Among the Riccia forms the thallose body produces sex organs and does chlorophyll work with no special differentiation of regions. From this condition there is evident a tendency to segregate the sex organs into definite regions, so that eventually the region of the body devoted to sex organs becomes quite distinct. The differentiation of a sex organ region is still further emphasized by its separation from the rest of the body by being carried up upon a vertical branch, an extreme case being displayed by Marchantia. As a result, the chief chlorophyll work and the production of sex organs are distinctly set apart by the organization of a gametophore arising from the thallus.

The gametophore, primarily a sex organ branch, proves to be more favorable for the display of chlorophyll tissue than the thallus, and the simple leaves of mosses appear, supplementing the chlorophyll work of the thallus. In sphagnums the thallose body continues associated with the leafy gametophore. In the true mosses, however, the chlorophyll work of the gametophyte is more or less given over to the gametophore leaves, and the thallus region is reduced to the so-called "protonema." In a very true sense, therefore, the gametophyte is always a thallus, special vertical or radial branches being developed in liverworts as gametophores, and in mosses as leafy gametophores. The loose habit of homologizing the leafy "moss plant" with a liverwort thallus on the one hand, and a fern prothallium on the other, is not merely bad morphology, but is apt to be very misleading.

The suggestion to be obtained from this history is that leaves may develop in response to more favorable conditions for their work, and such development may result in the great reduction of chlorophyll work done by the less favored region, and its consequent simplification. It is evident that with the exchange of an aquatic for a terrestrial habit the thallose body would not be a favorable type for chlorophyll work, and that the development of chlorophyll tissue upon erect structures of various kinds might follow. Among bryophytes the erect structure laid hold of is the gametophore, and not the sporogonium. I grant that this same reasoning would make the sporogonium of the Anthoceros forms a specially well adapted erect structure for the development of leaf tissue and hence leaves. The objection, however, is that the sporogonia of bryophytes are most persistently spore-bearing structures and nothing else, every tendency towards more complex organization having spore production and spore dispersal in view; and that such specialized structures are not apt to be productive of new lines of development.

In considering, therefore, whether it is possible to disregard the bryophytes in our search for the origin of the leafy sporophyte, we are largely influenced by the fact that the bryophyte sporophyte, throughout its whole history, is dominated by a tendency which does not appear in the pteridophyte sporophyte. Before the establishment of alternate generations the plant body may be said to have had three functions, namely, chlorophyll work, and the production of gametes and spores. The appearance of the bryophyte sporogonium was dominated by the separation of spore formation from the other functions, chlorophyll work being retained by the gametophyte, along with gamete production. Attention has been focused so long upon the gametes and spores as the two dominant factors in differentiation that it is hard to conceive of the possibility of the domination of another factor. It is entirely conceivable, however, that another form of differentiation may have occurred, dominated by the needs of the chlorophyll work, and not by spore production. Certainly a great need for change, when aquatic conditions were exchanged for terrestrial, was in connection with the display of chlorophyll tissue. It would seem as if the bryophytes had laid emphasis upon spore production, and therefore never became organized for the fullest use of terrestrial conditions; while the pteridophytes laid emphasis upon chlorophyll work, and became highly organized for terrestrial life. It would seem possible, therefore, with the three factors to take into account, that two distinct asexual lines may have been organized, distinct in the factor selected to dominate.

Such a conception may be simple enough, but it is hardly worthy of consideration without more practical statement. If more favorable structures can be developed in response to the needs of spores or gametes, there seems to be no good reason why more favorable structures may not be developed in response to the needs of chlorophyll work. If such a response in structure is possible, it would naturally express itself first in developing the largest display of chlorophyll tissue in the most favorable region of the body, which would gradually become differentiated more and more distinctly from the rest of the body. It does not seem clear why the appearance of an erect leafy axis, bearing neither gametes nor spores, is not quite as supposable as the appearance of a sporophore with neither gametes nor leaves, or a gametophore with neither spores nor leaves.

Of course such a leafy axis would be an integral part of the thallus body from which it was developed, and in no sense a distinct "generation," any more than the leafy gametophore and the protonema of mosses are distinct generations. Upon such a leafy axis spores would find a more favorable position than upon the ordinary thallus body, and eventually they would be segregated upon the leafy axis, developing in connection with chlorophyll tissue just as they had in the thallus body. In such conditions comparatively simple sporangia would be developed, being entirely subordinated to the nutritive tissues. A parallel case is found in the gametophore of mosses, which also prove favorable for leaf development; or even in the sporogonia of certain bryophytes, which also prove favorable for chlorophyll tissue, but this is rigidly subordinated to the work of spore production.

With the development of a leafy axis bearing spores, there is no reason why it should not become independent of the thallus body which produced it, as the leafy gametophore of mosses becomes independent of the protonema. The great difference in the final result in the two cases arises from the fact that in mosses the protonema is without gametes or spores; while in the case we are supposing the thallus body produces gametes, and the leafy axis spores. That a thallus body can directly produce just such a leafy axis bearing spores is testified to by the numerous cases of apogamy observed among pteridophytes. In fact, the theoretical life history we have been tracing is concretely represented by the life history of a fern in which apogamy has occurred.

If the phenomenon of apogamy represents the primitive status of the leafy sporoyhyte, it remains to imagine how this spore-bearing leafy axis could have become the usual product of the oospore. We find no trouble in believing that the usual oospore product frequently appears apogamously, for this has been demonstrated; but to imagine a general primitive apogamous habit of origin gradually passing into a predominant oospore habit of origin is difficult. In the condition supposed, namely, a thallus body producing gametes, and a special leafy axis bearing spores, zygotes and spores would have the same power, the germination of each resulting first in the thallus body and afterwards the leafy axis. If real alternation can be brought about by such a condition, the thallus portion of the zygote product and the leafy axis portion of the spore product must be gradually eliminated. In other words, the tendency would be to eliminate that particular region which is concerned in producing the reproductive body. Perhaps such a tendency is no more difficult to understand than the fact that a spore produces a gametophyte rather than a sporophyte, and a zygote produces a sporophyte rather than a gametophyte. A common explanation has been that a zygote, for some reason, stops reproducing the plant body which organizes it, and begins to produce an entirely new structure, which certainly seems to have been the case in the formation of the sporogonia of bryophytes. It would seem no more difficult for a zygote to stop producing one distinct portion of the plant body, and to continue producing the other.

Why in both cases it tends to produce the structure less immediately related to it, rather than the one which has originated it, is a question which cannot be answered at present. Cytology may offer certain suggestions, but they are vague as yet. The fact that the chromosomes are doubled in number by the process of fertilization, and are reduced again in the sporogenous tissue may have some bearing on the question. It seems clear that in all life histories where the sexual act occurs there must be a corresponding reduction division somewhere. In distinct alternation of generations, the "doubling" and the "reduction" are associated with the two generations. But before distinct alternation was established "doubling" and "reduction" must have occurred, and there is no present reason to doubt that in such case reduction often, if not generally, occurred in connection with the development of spores. When, therefore, the zygote was restricted to one region of the body, and the spore to a very distinct region, the alternation of "doubling" and "reduction" might well develop into an alternation of generations.

The very interesting results obtained by Strasburger and Farmer in their study of Fucus, which show that the reduction division in that plant occurs in connection with the development of the sex organs, may be correlated with the absence of spores. Such an observation emphasizes the fact that reduction must occur somewhere, and if sporogenous tissue is not developed, it would seem more likely to occur in gametogenous tissue, representing a new cell sequence, than in ordinary nutritive tissue.

With such an origin of the leafy sporophyte, it would follow that foliage leaves are not secondary, but primary structures, and that sporophylls have arisen from the differentiation of foliage leaves bearing sporangia, a state of things certainly suggested by the most primitive pteridophytes known. It would further follow that the evolution of the strobilus has followed the development of foliage leaves, a view in accordance with the older morphology. Such a view would make intelligible the great "gap" recognized as existing between bryophytes and pteridophytes, as the two groups would not be phylogenetically connected, and would have developed along very divergent lines from the first. It would mean that at least two independent sporophyte lines have appeared, the bryophyte line probably with an antithetic origin, and the pteridophyte line possibly with an homologous origin. The great prominence of the latter line, with its spermatophyte sequence, is correlated with the development of a vascular system, and it would seem as though the evolution of an elaborate vascular system must have depended upon the domination of chlorophyll work.

Perhaps one of the strongest arguments against the polyphyletic origin of archegoniate plants is the constant character of the archegonium. It would seem to some inconceivable that an organ so definite and so characteristic, and so unlike anything in thallophytes, could have appeared in two independent lines. However, the possibility of two independent appearances of such an organ would depend upon its origin, a subject of great obscurity. That it has been derived in some way from the oogonium of thallophytes seems hardly to be questioned, and that it is one of the results of the exchange of aquatic for terrestrial habits seems hardly less doubtful. That the archegonium represents a group of oogonia protected by a layer of sterile tissue seems to be a reasonable suggestion, and that the differentiation of this sterile protective layer into neck and venter would follow naturally from the exclusive functioning of the innermost oogonium seem probable enough. The conditions which induced this protection of aggregated oogonia, however, could hardly be claimed to have resulted but once in an archegonium.

It must be acknowledged that if the leafy sporophyte has had any such origin as has been indicated above there is no algal evidence that can be presented, as in the case of the leafless sporophyte. It must be remembered, however, although it may be regarded as a convenient refuge for all theories of phylogeny, that we are dealing with a structure whose origin is very ancient. Why the algæ continue to give suggestions as to the origin of the bryophyte sporogonium, and, so far as known, give no intimation of the independent origin of the leafy sporophyte, is a pertinent question. It seems to be also true, however, that the bryophytes give no clear suggestions as to the origin of the leafy sporophyte, and we are left to imagine the method of its origin from either group.

In thinking of this possible disconnection of the bryophyte and pteridophyte lines, it may be well to recall the similar experience of the gymnosperm and angiosperm lines. Certainly the gymnosperms and angiosperms seem to have more characters in common than do the bryophytes and pteridophytes, and seem to be more insistent in their demand for a common phylogeny; yet that the gymnosperms represent at least one independent phylum can hardly be longer doubted.

All such discussion is, of course, very vague and general, and may not commend itself to many as profitable. But it serves its purpose in stating the problem, and in presenting the possible alternative solutions. We have been in danger of restricting the operations of evolution too rigidly, making the lines of advance too few, and forgetting the possibilities of change during the enormous stretches of time. The polyphyletic origin of similar structures and of similar groups makes the problems of phylogeny immensely more complex, but is probably much more consistent with the facts.

The University of Chicago.

BRIEFER ARTICLES.

THE SEEDLINGS OF *JATROPHA MULTIFIDA* L. AND *PERSEA GRATISSIMA* GÄRTN.

(WITH SIX FIGURES)

TWO MODES of germinating are characteristic of the dicotyledons, with the cotyledons above or under ground. The first is undoubtedly the commonest. In this case the cotyledons, as a rule, are freed from the seed-coat and develop as two, seldom only one or several, leaf-like organs. In the other case, however, the cotyledons either remain enclosed in the seed or become free, but stay under ground. These are the principal forms of germination which Klebs[1] has ascribed to the dicotyledonous orders, and it is interesting to see that both forms may occur not only within allied genera, but even among species of the same genus. Furthermore, the germination itself exhibits a number of biological variations in regard to the relative development of the primary root, the hypocotyl, and the cotyledons. But it would seem very difficult to find any deviation from the rules given above, at least from the first, in which the cotyledons are above ground and free, while the second comprises two possibilities, enclosed or free cotyledons. When the seed-leaves are carried up above ground, this is not necessarily connected with the development of a hypocotyl, nor are the underground seedlings always destitute of such. But when a long hypocotyl raises the seed above the ground and the cotyledons, furthermore, are provided with petioles of considerable length, we should never expect to find the seed-leaves permanently enclosed in the seed and falling off without being exposed to sunlight. It would seem very strange if any such case really existed.

It was, therefore, very surprising to learn that Fr. Müller, in Blumenau, Brazil, had discovered a plant which exhibited this peculiar mode of germinating, the *bicuiba* or *Myristica Bicuhyba*, a tree of the primeval forests in Brazil.[2] The seedling of this tree possesses a long hypocotyl and two deeply lobed cotyledons, which, although above ground, do not leave the seed. To this instance may, however, be added a second, but of an order very remote from the Myristicaceæ. A few weeks ago Mr. G. W. Oliver, of the botanical garden at Washington, D. C., called my attention to some very odd-looking seedlings of *Jatropha multifida*, which were kindly submitted to the writer for closer study. The germination of this plant had, so far, only been very briefly mentioned by Sir John Lubbock,[3] who merely recorded it as an exception from that of the other Euphorbiaceæ. The seedling develops as follows.

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[1] KLEBS, GEORG: Beiträge zur Morphologie und Biologie der Keimung. Untersuch. aus d. Botan. Inst. Tübingen 1: 536–635. 1885.

[2] MÜLLER, FR.: Keimung der Bicuiba. Berichte d. deutsch. botan. Ges. 5:468. 1887.

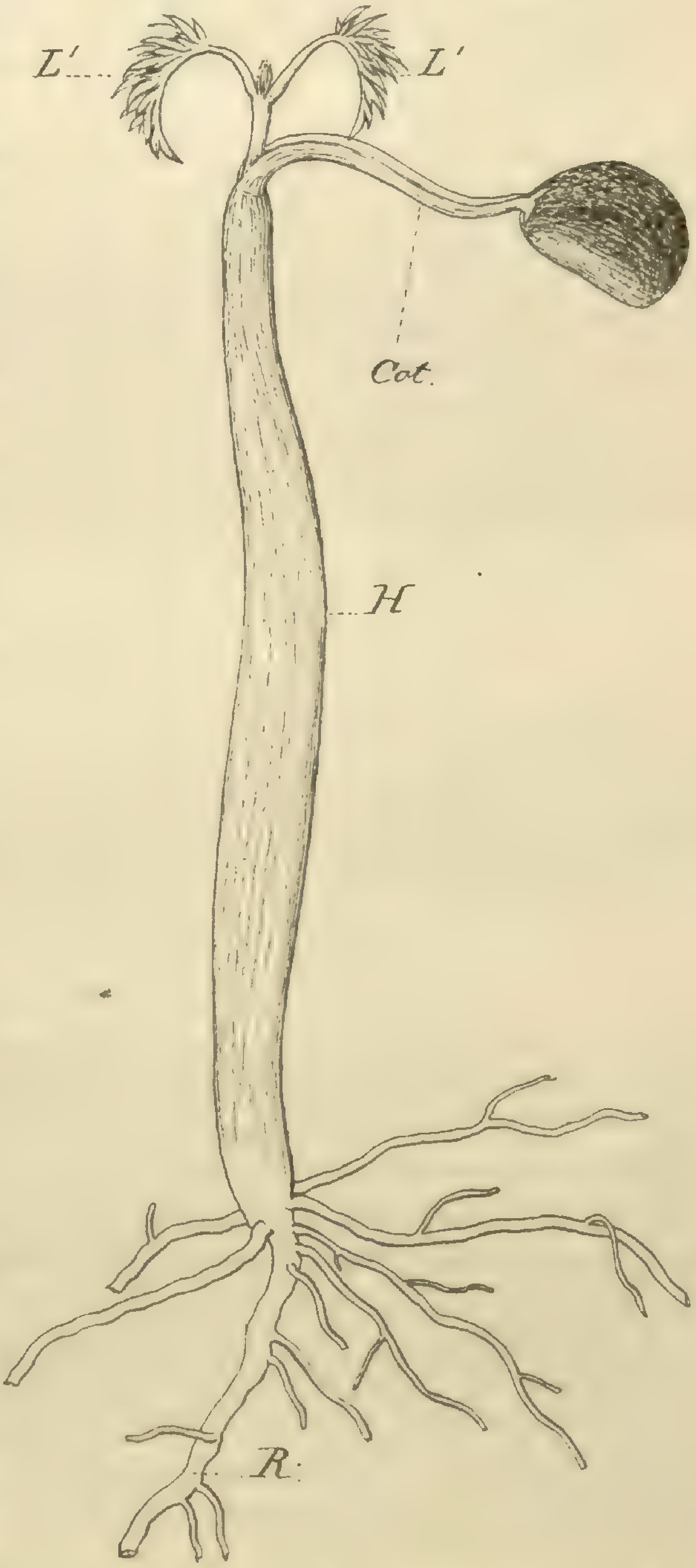

FIG. 1.—Seedling of *Jatropha multifida* L.; natural size.

Next to the primary root the hypocotyl increases very considerably in length and penetrates the soil by making a strong curvature until the seed becomes raised above ground, while simultaneously the petioles of the cotyledons have reached their final development and attained a very considerable length. While the hypocotyl increases rapidly in length and thickness, the cotyledons show no signs of becoming freed from the seed-coat. The plumule begins to develop a distinct internode, and the first leaves, being opposite and of approximately the same shape as the later ones, appear a short time before the cotyledons drop off, with their leaves still enclosed. The accompanying drawing (*fig. 1*) illustrates a seedling with the cotyledons, *Cot*, attached, and the first leaves, L^1, almost expanded. The primary root, *R*, is persistent and branches very soon, while a few secondary ones proceed from the base of the hypocotyl. The very long and thick hypocotyl, *H*, is green and glabrous; there is an early development of cork-layers; the bark-parenchyma is broad and traversed by a number of laticiferous ducts. The mestome bundles are of normal structure and between these are numerous strata of interfascicular cambium. A pith occupies the center of the hypocotyl; its cells are like those of the bark, thin-walled and filled with starch, but there are no laticiferous ducts. As stated above, the cotyledons have long petioles and their blades (*fig. 2*) are mostly oblong with a short point, but without any lobation as was observed in *bicuiba*. They are somewhat fleshy and pale in color; nevertheless, stomata are present on the upper surface and not on the lower. Along the ribs on the upper face, two kinds of hairs are visible. These are either long, multicellular, and pointed, or unicellular and almost globular in shape. The latter (*fig. 3*) are quite abundant in contrast to the first, and in some places they covered the surface just above the ribs. They represent glandular hairs, but their function could not be ascertained. The lower surface of the blades, which lie close up to the endosperm, is wholly glabrous. A typical palisade tissue was observed, covering a rather dense pneumatic tissue, and both contained abundant deposits of starch; laticiferous ducts were also very frequent.

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[3] LUBBOCK, J.: Seeds and seedlings.

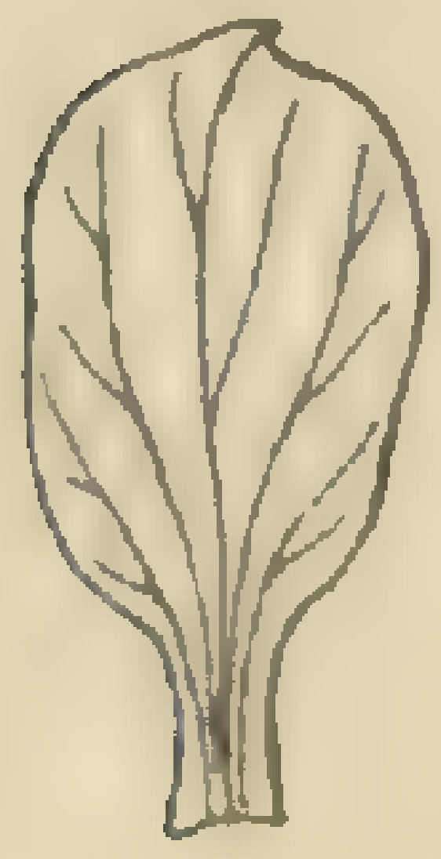

FIG. 2.—Blade of a cotyledon; natural size.

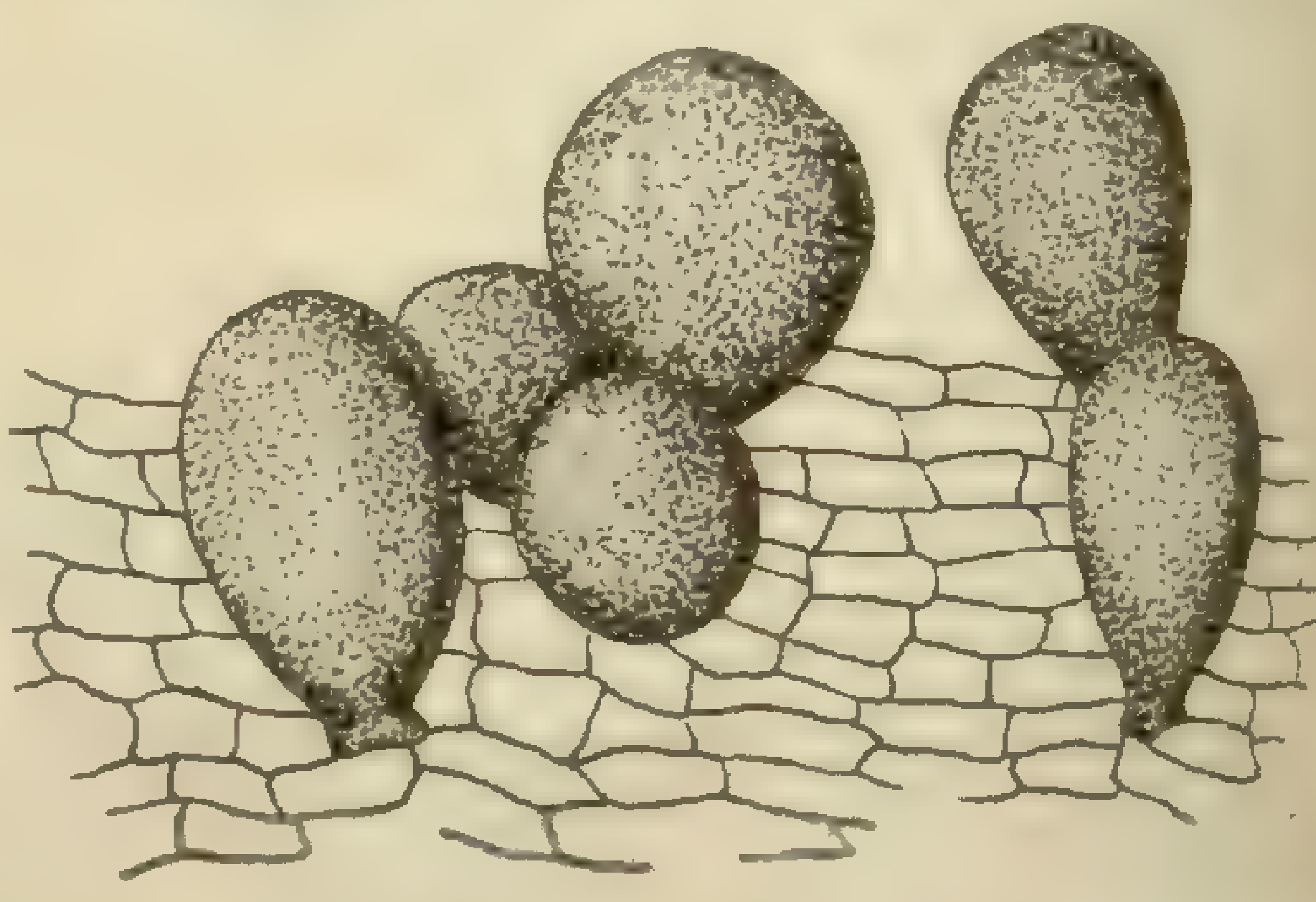

FIG. 3.—Epidermis with glandular hairs from the cotyledons of *Jatropha multifida*. × 240.

This seedling has no mate among the other species of Jatropha, which have been studied heretofore. Sir John Lubbock figures and describes *J. Curcas* L. and *J. podagrica* Hook., both of which possess stout and long hypocotyls; furthermore, their cotyledons are free and provided with distinct petioles, although not equaling in length those of *J. multifida*.

Another and very peculiar manner of germinating was observed in *Persea gratissima* Gärtn., of which several seedlings were cultivated at the same time in the botanical garden at Washington, D. C. It is strange that the Lauraceæ have been almost entirely passed by in works dealing with seedlings, none having been recorded either by Klebs (*l. c.*) or Sir John Lubbock (*l. c.*). Schacht[4] appears to be the only author who has given us some information about them; he states that the seed of *Persea gratissima* germinates while the fruit is still attached to the tree. This author observed, also, that in this species of Persea the plumule attains a very early development with a number of leaves, similar to Juglans and Tropæolum. Having detected a few other peculiarities connected with the germination of Persea, and having been unable to find any figure of this, I take this opportunity to publish and illustrate my observations, together with the still more remarkable case of germination just described.

In *Persea gratissima* there is no endosperm, and the large cotyledons remain enclosed by the seed-coat. No hypocotyl develops during the germination, but the plumule grows out very soon as a shoot with several leaves, while the primary root at the same time has attained a considerable length and developed a number of very strong lateral roots arranged in whorls of from three to five or more. In the accompanying drawing (*fig. 4*) the plumule has developed as a single shoot, and it is very strange to notice that the very first four leaves, L^1, are not only opposite, but even provided with petioles and blades, thus imitating the typical leaf of this species. On the other hand, the succeeding five or

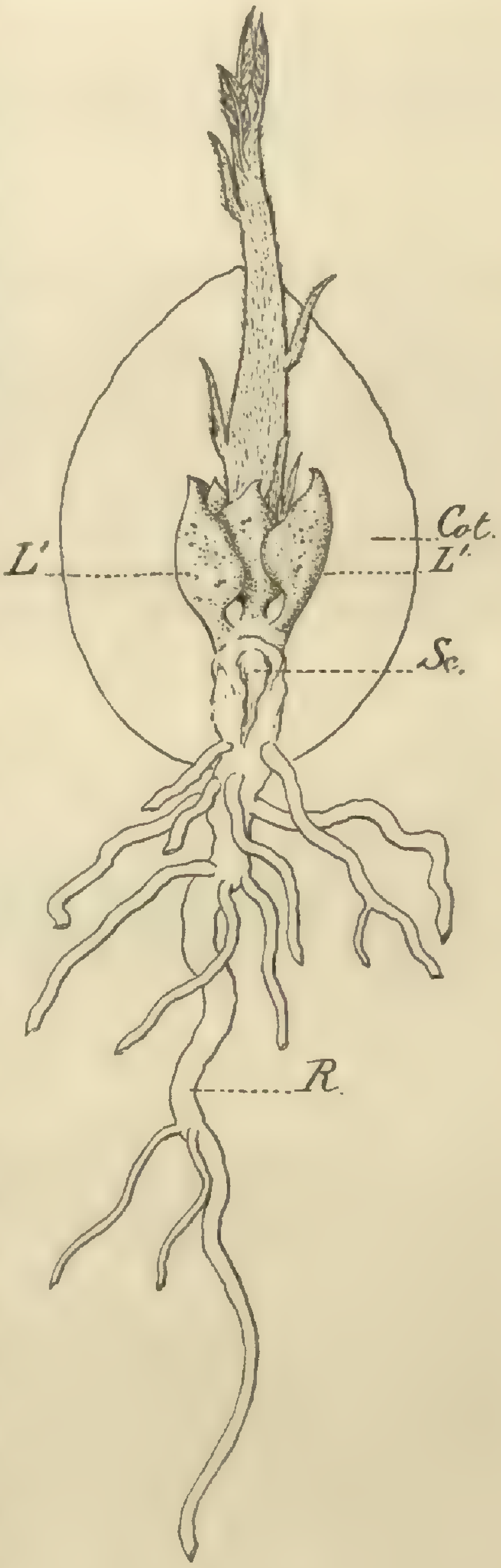

FIG. 4.—Seedling of *Persea gratissima* Gärtn., natural size. *R* primary root; *Sc*, scar from the removed cotyledon; *Cot*, the other cotyledon; L^1, the first leaves.

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[4] SCHACHT, HERMANN: Lehrbuch der Anatomie und Physiologie der Gewächse 2: 447, 468. 1859.

six leaves are almost scale-like, with no petiole or blade, but covered with silky hairs like the axis and the proper leaves when young. Thus *Persea gratissima* demonstrates the fact that in seedlings with enclosed cotyledons, there may be an alternation of various forms of leaves, while in Juglans and Carya, for instance, all the first leaves are scale or bristle-like.

When, however, the plumule does not develop as a single shoot but as a complex of ramifications, the first leaves become almost suppressed and appear only as small and rather broad scales (*fig. 5*). In this case, the shoots have pushed out very freely from the axils of the lowest leaves, and have almost attained the same development as the plumule itself, possessing elongated internodes and some narrow scalelike leaves with silky hairs. Besides these lateral branches, there is a bud observable in the axil of each of the two cotyledons (*fig. 6*), which is evidently ready to develop if the mother-shoot, the plumule, should become injured. The first developed leaves were perfectly glabrous, in contrast to the succeeding ones. There were a few stomata on the lower surface, but none on the upper. The mesophyll formed a homogeneous tissue filled with starch, and there was no indication either of collenchyma or stereome above or underneath the mestome bundles.

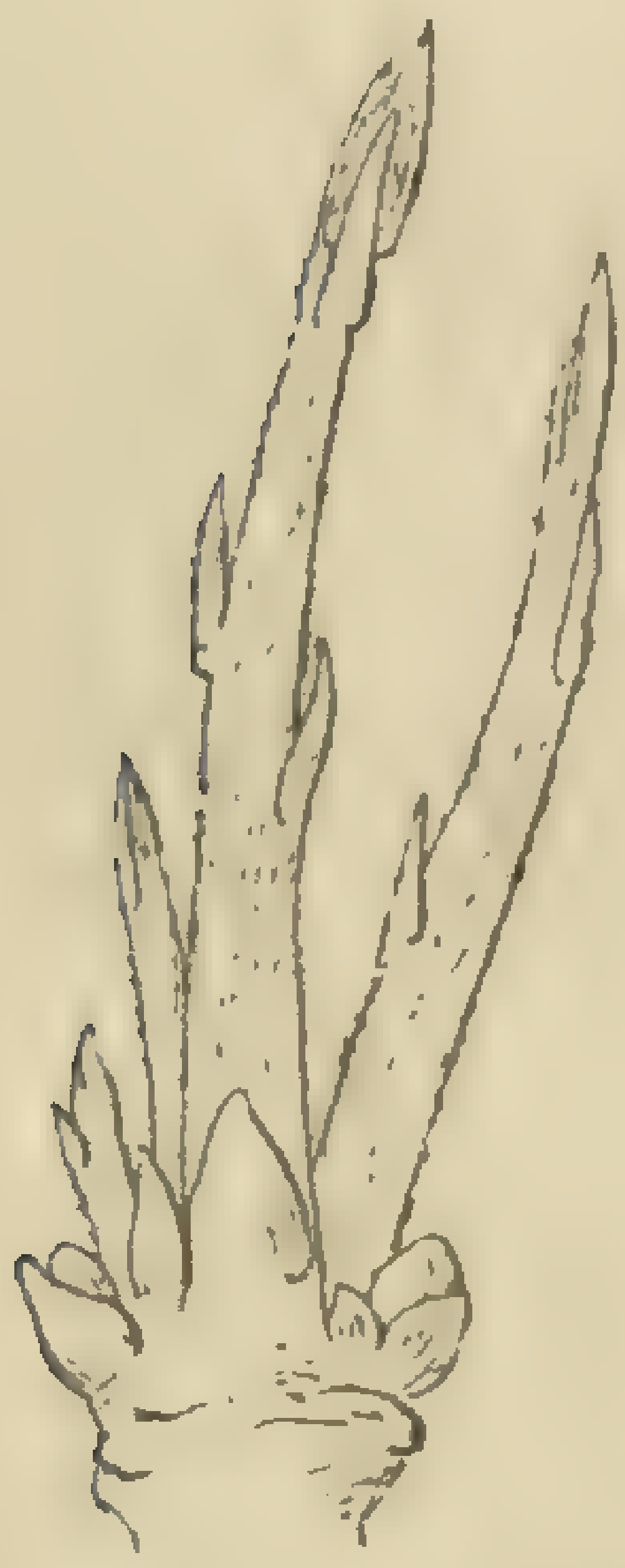

FIG. 5.—Plumule of seedling of *Persea gratissima*; natural size.

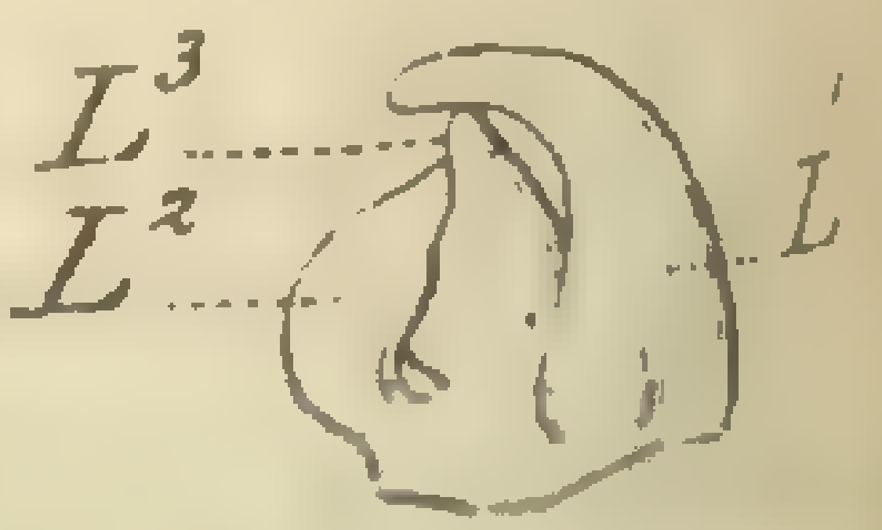

FIG. 6.—A bud from the axil of a cotyledon of *Persea gratissima*. L^1, L^2, L^3, the first, second, and third leaf; magnified.

In Lindera and Sassafras, at least in their North American representatives, the germination takes place underground, but the plumule develops as a single shoot. The first leaves are bristle-shaped, succeeded by a few whose shape is approximately the same as that of the typical leaf. The three-lobed leaf of *S. officinale* Nees, however, is only seldom observed in the first year of the seedling, those developing at this stage being ovate and entire.—THEO. HOLM, *Brookland, D. C.*

ON THE BLIGHT OF SORGHUM.

THE tissues of the different organs of sorghum, under certain conditions which are not as yet perfectly known, may become the seat of an intense production of red pigment which impregnates them. The cells die and disintegrate, the disease being known as sorghum blight (*sorgho brûlé*, *Hirsebrand*).

The disease was first described in Italy by Palmeri and Comes,[1] who have attributed it to the development of Saccharomycetes and bacteria. Later, in America, Burrill (1887) studied anew the blight, and, after having isolated sporogenous bacteria from the infected tissues, he attempted the inoculation of healthy plants. The results were variable; however, the author inferred the parasitism of the *Bacillus Sorghi*, nov. spec., and prescribed some measures for the preservation of plantations from the invasion of the disease. Analogous experiments by Kellerman and Swingle[2], more convincing than the preceding, because of the number of infections obtained, led to the same conclusions. More recently, Bruyning[3] has examined some plants of sorghum attacked by the blight, and formulated some conclusions different from those of the preceding authors. After having isolated several bacteria from the red tissues Bruyning retained two species which he considered the only factors of the fermentative disease, but these species are chromogenous outside the sorghum. These microbes acted symbiotically, superposing their respective pigments, the one yellow, the other red, the mixture giving the coloration observed in the tissues. These views were not supported by any experiment of inoculation.

I have recently studied some specimens of blighted sorghum coming from Algeria.[4] I was able to convince myself that in this case the phenomena of the blight were caused by the parasitic development of yeasts in the tissues of the plant.

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[1] Notizie preliminari sopra alcuni fenomeni di fermentazione del Sorgo saccarino vivente (Accad. d. Sc. fis. e mat. di Napoli, fasc. 12, 1883).

[2] Report of Bot. Depart. of the Kansas Stat. Agri. Coll. 1888.

[3] La brûlure du Sorgho, etc., et les bactéries qui la provoquent (Arch. Néerland. 4e et 5e livr., pp. 297–330. 1898).

[4] These infected stems were sent to the botanical laboratory of the School of Pharmacy by Professor Trabut. Professor Guignard had the kindness to entrust me with the examination of them.

Already, by microscopic examination, it had been ascertained that in many regions of the central parenchyma of the stem the cells and the intercellular spaces enclosed masses of a small ovoid yeast, measuring from 1.5 to 2.5μ on an average. It was, moreover, the only microorganism which could be directly observed, even with great magnification and with the aid of staining reagents.

It was easy to isolate this yeast in a pure state by taking up the germs with a sterilized needle from the center of the stem, the section being made with a flamed scalpel, or by removing small cylinders of the red tissue from the pith by means of a sterilized trocar. The first culture liquid was a 5 per cent. glucose bouillon. Some subsequent isolations by means of Petri dishes with bouillon sugar gelatin have given, to the exclusion of all other organisms, white colonies formed by a yeast morphologically identical with that which had been observed in the diseased sorghum.

Cultivated on the surface of carrot, potato, and on different gelatin or gelose media with sugar, the yeast grows slowly in creamy-white colonies. It apparently keeps the same dimensions as those observed in the tissues of the plant. Sown in unfermented grape juice, or in various artificially sweetened liquids suitable for the culture of yeast, this organism shows a feeble alcoholic fermentation. The fermenting power is slightly increased by a series of re-sowings in the same medium. My attempts to obtain ascospores have been fruitless, and I am not able at present to classify this yeast with the true Saccharomyces.

The mere fact of isolating a yeast from the tissues of the blighted sorghum is not sufficient to enable one to conclude that this yeast is a parasite, and that it brings about the symptoms of the disease. It is known, in fact, that a large number of these ferments may be encountered on the surface of stems and leaves; it would not be astonishing, therefore, if some dead tissues, still containing in their cells a part of their reserve sugar, were invaded by the saprophytic development of a superficial yeast. The following experiments, however, show that the yeast isolated from the diseased sorghum does develop in the healthy tissues of the plant, and in them brings about the phenomena of the blight.

Sorghum plants raised from seed and cultivated in a hothouse during the months of November and December 1898 and January 1899 were inoculated with pure cultures. All aseptic precautions were taken to avoid the introduction of foreign microbes. I used Dr. Roux's sterilizable syringe with a fine injection needle, in order to reduce to the minimum the wound necessary for the introduction of the culture. Only the stems were inoculated. The epidermis was exposed by cutting a small flap in the leaf sheath, carefully disinfecting by means of a red-hot iron, and, after the inoculation, the opening sealed by means of warm wax or sterilized paper. The plants experimented upon were successively examined at intervals of time varying from five days to two months. In every case the yeast had developed and multiplied in the intercellular spaces, and in the interior of the living cells to a distance of 10 to 15^{mm} above and below the point of inoculation.

The microscopical appearance was that which may be observed in the tissues of sorghum spontaneously infected ; the lesion was also rendered visible in the same way by the red coloration of the parenchyma and fibrovascular bundles. These latter transfuse the coloring matter the whole length of the internode, and quite beyond the infected region, so that the appearance of the pigment at a certain point of the tissue is not a sure sign that the parasite is present at that point. On the surface of the stem may be observed long red lines corresponding to the outer bundles and their contiguous parenchyma seen through the transparent stem.

In these experiments, the isolation of the parasite from the affected pith has shown, by comparison with the control, that the parasitic yeast was the same as that in the cultures used as a starting point.

It is probable that the reserve sugars of the cells of the sorghum constitute the principal food of the parasite. Unfortunately the volume of infected tissue, limited and especially difficult to separate out, has not permitted the changes carried on by the parasite in the chemical composition of the plant to be estimated in this particular.

Are other yeasts capable of producing in the sorghum similar phenomena of parasitism ? The following experiments answer in the affirmative. Inoculations have been made aseptically in the stems of healthy sorghum with pure cultures of wine yeast (round yeast of champagne [Bowzy]). The parasitism has been established under the same conditions as before, the yeast developing in the intercellular spaces and in the pith cells of the stem with the accompanying production of the characteristic red pigment, and with transfusion by the bundles of the internode.

On the other hand, what is the origin of the pigment? It is a general observation that wounds inflicted on the tissues of the sorghum developed a red coloration around the injured part. It is important to understand precisely in the preceding experiments the rôle of the local lesion induced by the inoculating needle. Aseptic punctures in the pith of healthy sorghum stems were made under conditions identical with those of the preceding experiments, the inoculating fluid alone being omitted. Under these conditions the pigment appeared in the wounded cells, but it was not abundant and was rigorously confined to the wound. The quantity of coloring matter thus produced is not capable of being carried by the bundles and of spreading beyond the actual point of the lesion. The experiment shows, however, that the chromogenous property belongs to the wounded cells of the sorghum.

From the preceding facts it may be concluded:

1. That yeasts may develop in the living cells of the sorghum.

2. That the parasitism of these yeasts may bring about an intense red coloration of the plant tissues, this coloration being the same as that which may be observed in the disease of sorghum called the *blight*. The production of the pigment appertains to the affected cells, and the parasite takes part only through the lesion which it produces.

These results confirm the old hypothesis of Palmeri and Comes, who, observing the fermentative phenomena of the red juice of the pith of the blighted sorghum, had inferred from it the parasitic action of the Saccharomycetes without giving it experimental proof. The same facts, moreover, are not contradictory of the experiments of Burrill, Kellerman, and Swingle. In fact, it may be concluded that, the red coloration being the result of a chromogenous function characteristic of the wounded cells of the plant, different parasites, yeasts or bacteria, may, by developing in the tissues, induce by continued lesion the production of a considerable quantity of the pigment.

On the contrary, it is necessary to make complete reservations as to the conclusions of Bruyning, who, attributing to the bacteria themselves the chromogenous function, denies to all micro-organisms lacking this function while outside the host plant the power of inducing the phenomena of sorghum blight.—MAXIME RADAIS, *School of Pharmacy, Paris.*

ROOT SUCKERS ON DOUGLAS FIR.

The occurrence of stool shoots among deciduous species of trees is very common, and their production by the forester is resorted to for the reproduction of many species. Among conifers the formation of stool shoots is limited to a few species. The California redwood (*Sequoia sempervirens*) is very commonly reproduced in this way. Much less common than stool shoots are root shoots, often known as root suckers or suckers. Among conifers only the redwood, the California nutmeg (*Tumion Californica*), and the short-leaf or yellow pine (*Pinus echinata* Miller) have so far been reported as producing root suckers.

For several years the woodsmen of western Washington have recognized in the fir forests a curious growth which they have called "sap suckers." As we see them in the forest they appear as a broken stub ranging in height from 0.6 to 3.5^{m}. Without leaves or branches, they appear entirely lifeless until cut into with an axe. An examination shows that they are covered with living bark and beneath that a living woody tissue very hard and with a grain of very fine and intricate burl.

The sap suckers vary greatly in size and external appearance. The diameters range from 30 to 60^{cm} and the height from 0.6 to 3.5^{m}. Investigation has shown that they are connected with one of the main roots of the parent fir, their distance from the parent trunk varying from 0.6 to 4.5^{m}, an average distance being about 1.8^{m}. The living bark bears little resemblance to the bark of the fir except in color. The clefts are much finer and the plates much smaller. The living wood, in every instance, forms only an enveloping sheath about a decaying stub. It varies in thickness from 12 to 50^{mm} and there is great diversity in the disposition of this living sheath. It begins at or below the surface of the ground and grows upward, entirely encircling the decaying core. In many cases where the stub was very short the wood burl entirely covered it over, forming sometimes a low globular protuberance about 0.6^{m} high and sometimes a column 3.5^{m} high; but in the great majority of cases the burl covering has extended to only a portion of the height of the stub, so that the rotting dead end still protrudes above.

The "sap sucker" is only a secondary growth on an ordinary root sucker. The formation of these on the roots of the Douglas fir, *Pseudotsuga taxifolia*, was proven by examination. In the dense woods of this region it is hard to distinguish the suckers from ordinary seedlings. Like most seedlings, those of the fir are very weak, and when they reach a maximum diameter of about 25^{cm} they die and begin to decay. At this time the formation of the secondary burl covering begins. The nourishment before given the sucker is utilized in covering over the dead and decaying stub with this new live growth.

In one instance a dead sucker had been covered with a primary burl covering to a height of 1.5^{m}. This had died, the bark had fallen off, and a second burl covering, still alive, had covered this over to a height of 0.9^{m}.

These suckers are found only in the moistest and densest forests. Even under the proper conditions they are very rare. They were observed in the summer of 1898, ten to fifteen miles from tide water, Chehalis county, Washington.— FRANK HAINES LAMB, *Biltmore, N. C.*

CURRENT LITERATURE.

BOOK REVIEWS.

General physiology.

THE RECEPTION by both European and American biologists of Verworn's *Allgemeine Physiologie* upon its publication late in 1895 was very cordial. The book awakened so widespread an interest that within three years a second edition was issued. This second edition has now been translated and edited by Dr. Frederic S. Lee, of Columbia University.[1] In its English dress the book will doubtless commend itself still further to English readers. Certainly it will put within reach of general readers a work that will give a better idea of the scope of physiology, and one that will present to special students readable and stimulating discussions of physiological problems.

The work may be called a treatise upon "general" physiology only in the peculiar sense that it discusses the general as opposed to the special functions of the cell. It does not deal with the functions of organs at all. On the whole the phrase *general cell physiology* would seem to describe it better.

It is a pleasure to find an animal physiologist who is yet to a reasonable degree familiar with plant physiology. But it is not for the views of plant function nor even for the facts adduced that the book may be commended to botanists. In these fields, indeed, one feels that Professor Verworn is traversing rather unfamiliar ground, in which he sometimes loses his way, to the leading astray of the unwary. Nevertheless the ability and suggestiveness of the book make it one which botanists inclined at all to physiology will do well to read. Professor Verworn has a luminous way of saying things, and while what he says sometimes suggests what not to say, more often his apt applications indicate others which he has not expressed.

The subjects treated, after an interesting introductory chapter on the aims and methods of physiological research, are as follows: living substance; its composition and the differences between living and lifeless substances; elementary vital phenomena, namely the phenomena of metabolism, of the changes of form, and of the transformation of energy; the general conditions of life, including a discussion of the origin of life and the history of death; stimuli and their actions; and the mechanism of life.

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[1] VERWORN, MAX.—General physiology, an outline of the science of life. Translated from the second German edition and edited by FREDERIC S. LEE. 8vo. pp. xvi+615. *figs. 285*. London and New York: The Macmillan Company. $4.00.

No detailed discussion of the book need be entered upon, since it is already fairly well known in the original form. The translation has been admirably done by Professor Lee. The smooth and readable German of Verworn has been converted into easy and idiomatic English.—C. R. B.

Some popular books.

ALICE LOUNSBERRY is the author of *A guide to the wild flowers*[2] recently published. Mrs. Ellis Bowan has had charge of the illustrations, which consist of sixty-four colored and one hundred black and white plates, and fifty-four diagrams. Dr. N. L. Britton has written a brief introduction. The numerous attempts to provide easy and interesting ways of recognizing plants indicate a real demand and one that is very hopeful. Nature study is finding a prominent place in the schools, and any book which stimulates it properly is to be commended. The plants selected for this book are well illustrated and fairly well described, and should be recognized easily by the intelligent observer. Although strictly taxonomic, the plants are presented in ecological groups; as for example, "plants growing in water," "plants growing in moist soil," "plants growing in dry soil," etc., etc.—J. M. C.

ANOTHER RECENT book intended to encourage Nature study is *Field, forest, and wayside flowers*,"[3] by Maud Going (E. M. Hardinge). It professes to be "untechnical studies for unlearned lovers of nature," and is a charming book. The author does not deal in the usual fairy tales, but evidently knows what things are and what they are for, so far as current knowledge goes. The titles of the chapters are suggestive of the topical character of the book. A few of them are as follows: crocuses, dandelions, the flowering of the forest trees, green leaves at work, grasses, climbing plants, a handful of weeds, in winter woods, etc. The photographs are especially excellent, and some of our common plants are made to stand out with remarkable distinctness. The book can be commended to all those who wish to come into contact with nature in an untechnical way, and also to teachers in charge of nature study.—J. M. C.

EDWARD KNOBEL has attempted to make the identification of the grasses, sedges, and rushes of the northern United States an easy matter.[4] The plates are numerous, and one of the features of the book is the series of marginal illustrations of essential structures opposite each description. Just how easy this will make the determination of species in these perplexing groups can be known only after experience. It is a question whether anything in clearness has been gained by substituting ear and earlet for spike and spikelet.—J. M. C.

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[2] A guide to the wild flowers. 8vo. pp. xvii + 347. New York: Frederick A. Stokes Company. 1899.

[3] Field, forest, and wayside flowers. 8vo. pp. xvi + 411. New York: The Baker & Taylor Company. 1899. $1.50.

[4] The grasses, sedges, and rushes of the northern U. S. 8vo. pp. 78. *pl.* 25. Boston: Bradlee Whidden. 1899. $1.00.

A NEW NATURE READER has been provided by Kate Louise Brown, under the title *The plant baby and its friends.*[5] While the title suggests that the author has concerned herself with plant seedlings, a reading shows that the adult plant has received most consideration. Facts concerning many common plants are presented in the form of stories intended to be used as supplementary matter with younger children; and for such purpose the book should find a place. Some objectionable features of many books intended to assist children in nature study are happily absent from this book. Among these may be mentioned the treatment of fertilization and nomenclature. The stories concerning pollination are told in such an interesting way that even the youngest pupils should be interested. In some cases attempts at coloring as well as attempts at drawing lessons in morals from the plants studied have done violence to the facts, and the unfounded story of Egyptian mummy wheat is told once more. But aside from a few such errors it should be said that the plan and spirit of the book will make it very helpful with younger pupils.—OTIS W. CALDWELL.

FREDERICK LEROY SARGENT has produced an interesting little book for young people entitled *The corn plants, their uses and ways of life.*[6] Of course the phrase "corn plants" means cereals. The first pages are devoted to the mythology of the cereals, embracing the stories of classical antiquity to which allusions are so frequent in more modern literature.

For the botanist the chief interest is found in those pages which discuss the ecological adaptations of these important plants. A great deal of attention is given to such questions as protection against wind, weight, and excesses of moisture and dryness, several new drawings illustrating the structures which assist the plants in these respects. Few plants seem to be more interesting from the ecological standpoint, when one considers that they are grown in exposed places, absolutely unassisted by close relation with other plants which might serve to modify the effect of the wind or the sunlight.

In the section on the advantages of cereals as food-plants the author gives interesting descriptions of the methods of using cereals and the extent and geographical distribution of the various sorts. The book is full of good information put in an attractive style and should find abundant welcome.—O. W. CALDWELL.

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[5] The plant baby and its friends. 8vo. pp. 155. Boston: Silver, Burdett & Company. 1899. 48 cents.

[6] The corn plants, their uses and ways of life. 8vo. pp. v + 106. *figs. 32.* Boston: Houghton, Mifflin & Company. 1899. 75 cents.

MINOR NOTICES.

THE THIRD FASCICLE of "Illustrations de la Flore du Congo," by Wildeman and Durand, has just appeared, containing twelve plates, with descriptive text. The plates are exceedingly handsome, and their number has now reached thirty-six.—J. M. C.

A NEW classification of the Leucobryaceæ is proposed by M. Jules Cardot in *Revue Bryologique* **26**: 1–8. *pl. 1.* 1899. It is based chiefly upon the anatomical characters of the leaves as shown by cross-sections, such as the presence or absence of sclereides, and the form and arrangement of the chlorophyllose cells.—C. R. B.

SOME RESEARCHES of Loeb upon the influence of alkalies and acids upon embryonal development and growth[7] led to results which may have important applications to the growth of plants. He finds that weak alkalies (even .006% NaHO) accelerate the development and growth of larvæ of Arbacia (a sea-urchin) and the embryos of Fundulus (a fish), while weak acids retard. The cause of these actions is to be sought in the effect of the reagents on the oxidative processes of the protoplasm.—C. R. B.

NOTES FOR STUDENTS.

GEORGE J. PEIRCE has been studying the nature of the association of alga and fungus in lichens.[8] Speaking of the algæ he says that "it is neither logical nor sensible to conclude that their unusual position is beneficial to them," as free algæ can thrive, at least for a time, wherever lichens can. "There is no proof that algal cells serving as lichen gonidia are any better off as to food, protection, or situation than the average free algal cells of the same species." Of course the fungus is found to be absolutely dependent upon the alga. The author also affirms that the central body of the gonidial cells of Ramalina, Usnea, and Sphærophorus, is a nucleus, not a pyrenoid.—J. M. C.

HERMANN VON SCHRENK[9] has been investigating a disease of *Taxodium* known as peckiness, and also a similar disease of *Libocedrus decurrens*. In both cases the wood is destroyed in localized areas, which are surrounded by wood apparently sound. In both, also, a fungus mycelium was found, which flourishes within the diseased centers, but no characters appeared by which it could be determined. It is interesting to note the fact that these two trees, similarly affected, are the representatives of a race largely extinct, and grow in different parts of the country.

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[7] Archiv f. Entw.-mechanik der Organismen 7:631–641. *pl. 1.* 1898.

[8] Proc. Calif. Acad. Sci. III. **1**: 207–240. *pls. 41.* 1899.

[9] Eleventh Ann. Rep. Mo. Bot. Gard. 1–55. *pls. 6.* 1899.

The same author (*op. cit.* 6–70) has also discovered a peculiar sclerotioid disease of beech roots. Small tubercles were found attached to the fibrous roots, and upon examination these were found to be a twisted and contorted mass of rootlets invested by a sheath. It was impossible to determine the fungus. but it seemed probable to the author that it is distinct from those forming the mycorhiza.—J. M. C.

MESSRS. FARMER and WILLIAMS have continued their contributions to the life-history and cytology of the Fucaceæ by publishing a second paper[10] which deals with the development of the oogonia and oospheres, the phenomena of fertilization, and the early segmentation stages of the oospore, especially in the genera Fucus, Ascophyllum, and Pelvetia. The more interesting subjects discussed are the reduction in the number of chromosomes, the appearance and behavior of centrospheres and centrosomes, the formation of the walls of the oogonia, oospheres, and germinating oospore, and the part played by mucilage in bringing about the extrusion of the oospheres.

The reduction of chromosomes occurs in the papilla which is cut off to form the rudiment of an oogonium. This was also shown by Strasburger to be the case in Fucus. In the three successive mitoses by which the eight oosphere nuclei are formed, the reduced number of chromosomes is retained. Prominent centrospheres in the form of converging cytoplasmic radiations are present in every mitosis, whether of the oogonium or segmenting oospore. These could not be traced back to preexisting centrospheres, but appeared to arise independently by the activity of the protoplasm, and to disappear again after the mitosis. Though the centrospheres are cytoplasmic, the spindle between them is intranuclear in origin. Centrosome-like structures were sometimes seen within the centrospheres, but their number was not constant. There was no indication that the centrospheres of the first oosporic division are derived from the spermatozoid.

So few cases of polyspermy were observed that it is certain but one spermatozoid normally enters an oosphere. The existence of a wall around the newly fertilized eggs was conclusively demonstrated ; such eggs burst when transferred from salt water to fresh, whereas unfertilized eggs similarly treated merely swell up but do not burst.

Some interesting features with regard to fertilization were observed in Halidrys. Groups of gyrating spermatozoids were seen distributed over the surfaces of the oospheres, each attached by one cilium. Suddenly the spermatozoids leave the egg "like a crowd of startled birds," the egg at the same time becoming covered with conical projections. The authors consider this phenomenon as marking the moment of fertilization, and as a proof of a definite repulsion of the supernumerary spermatozoids.— WILSON R. SMITH.

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[10] Phil. Trans. Roy. Soc., London, B. 190 : 623–645. 1898.

ALTHOUGH the morphology of the embryo sac is still a very live subject, it is well to study its physiological relations, as has been done by Mlle. Goldflus in the ovules of a large number of Compositae. She discusses especially the function of the antipodal cells and of the so-called epithelial layer.[11]

It is well known that, after the rupture of the nucellus, the embryo sac in this family lies in contact with the epidermis of the inner portion of the ovule. The cells of this layer frequently become enlarged and beautifully columnar, and for this reason Hegelmaier called the layer the "endodermis," ascribing to it a protective function. Schwere, without committing himself in regard to its function, changed the name to "endothelium." The author revives the term "epithelium," previously used by Warming and Goebel, assigning to the layer a digestive function, as did Guignard.

The antipodal cells, variable, as usual, both in number and size, are said to be digestive in some plants, and in others, conductive in function. Their densely staining protoplasm and their tendency to burrow back into the "pseudo-chalazal" region are cited in support of this view. Moreover, the cells of the inner part of the integument are arranged in rows converging from all sides toward the sac, this structure being well adapted for the conduction of nutriment to the embryo sac and its embryo.

The author has evidently fallen into the error of supposing that, because the *function* of the antipodal cells is now discovered, the view of Strasburger and others regarding their *homology* is no longer tenable. One would naturally expect a prothallial region of a gametophyte to nourish the reproductive region, even to digesting the food if necessary. Hence the two theories, instead of being mutually hostile, are in perfect harmony. Would the author say that the "epithelial layer" is not epidermis, merely because it has here a special function, that of digestion?

In reviewing the article by Chamberlain on *Aster Novae-Angliae*, the author rightly challenges an unguarded suggestion about a "macrospore in an unusual position," but closer reading would have shown that he was merely comparing the free nuclear division in the large antipodal cell with the same process in a macrospore; and it would also have become evident that he was not confounding the two terms "oosphere" and "macrospore."

[11] GOLDFLUS, MLLE. MATHILDE : Sur la structure et les fonctions de l'assise épithéliale et des antipodes chez les Composées. Journ. de Botanique **12** : 374–384. 1898; **13** : 9–17, 49–59, 87–96. 1899.

We cannot help wishing that the author had given complete drawings of some of the more striking ovules, such, for example, as *Dahlia gracilis*, with the cell contents accurately shown. When a point is proven "by the appearance of the protoplasm, and the distribution of starch and other foods in the ovule," one quite naturally expects in the drawings definite delineation. In this, however, the six plates are sadly lacking.

The author has touched an important and suggestive field. Results from a comparative study of other groups ought to be easily accumulated from the many permanent preparations of ovules and embryo sacs in the possession of various botanists, which might repay reexamination from this new point of view.— W. D. MERRELL.

NEWS.

Dr. M. Treub, director of the botanical gardens at Buitenzorg, has been elected a member of the Royal Society of London.

Mr. H. G. Timberlake, instructor in botany in the University of Michigan, has accepted a corresponding position in the University of Wisconsin.

A biographical sketch, with portrait, of the late Edward Lewis Sturtevant is published by C. S. Plumb in the tenth report of the Missouri Botanical Garden.

Mr. Ernst A. Bessey has been appointed assistant in the Division of Vegetable Physiology and Pathology of the United States Department of Agriculture.

M. Ed. Prillieux, the eminent French phytopathologist, has been elected a member of the French Academy of Sciences, in the room of the late Charles Naudin.

Professor W. A. Setchell and other botanists of the University of California are about to leave on an expedition to study the flora of the Aleutian islands.—*Science*, June 23.

Professor P. H. Rolfs has been appointed professor of botany at Clemson College and botanist to the Agricultural Experiment Station of South Carolina, to succeed Dr. A. P. Anderson.

Professor Henry G. Jesup, for twenty-two years professor of botany in Dartmouth College, Hanover, N. H., has resigned, and Mr. G. T. Moore, of Harvard University, has been appointed instructor in botany.

The proprietors of *Nature* announce that they are about to reissue Sowerby's *English Botany*, third edition with supplement, containing descriptions and life size figures of every British plant, hand colored. The price varies from 16 to 19 guineas, according to binding.

The instructors and fellows of the University of Chicago who have received botanical appointments for the coming year are as follows: Dr. O. W. Caldwell, professor of botany, State Normal School, Charleston, Ill.; John G. Coulter, assistant in charge of botany, Syracuse University; Florence May Lyon, assistant in botany, Smith College; Dr. W. D. Merrell, instructor in charge of botany, University of Rochester; Herbert F. Roberts, assistant in Shaw School of Botany; Dr. Wilson R. Smith, instructor in charge of botany, McMaster University, Toronto, Canada.

THE BOTANICAL SEMINAR of the University of Nebraska is pushing work on the survey of the state and hopes to send additional parts of the "Flora" to the printer before the year is over. The Regents of the University made a small grant for a printing fund for the survey at their last meeting.

BY THE WILL of the late Professor O. C. Marsh, Yale University is given his fine residence with the extensive grounds and greenhouses for a botanic garden. The house may be used as the residence of the director, or as a botanical laboratory. It is to be hoped that this gift will stimulate Yale to develop its botanical work more worthily.

DR. EDWARD A. BURT, through the courtesy of Dr. W. G. Farlow, has been studying the Thelephoraceæ of the Curtis herbarium and published exsiccati in the cryptogamic herbarium at Harvard University. He expects to examine types and authentic specimens at Kew during July, spending the remainder of the summer in Sweden collecting and studying fleshy fungi.

AT CORNELL UNIVERSITY: Mr. W. A. Murrill has been appointed for the year as assistant cryptogamic botanist to the Experiment Station, to take charge of the work of Dr. B. M. Duggar during his absence in Europe. Messrs. Heinrich Hasselbring, Judson F. Clark, and George J. Hastings have been appointed assistants in botany. Dr. K. M. Wiegand has been promoted to an instructorship in botany.

AT THE UNIVERSITY OF MICHIGAN: Professor V. M. Spalding, who has been in California during the year, has returned to the East and will resume his work next autumn. Professor F. C. Newcombe will spend the summer in Paris to study the scientific institutes of that city. Dr. J. B. Pollock will have charge of the botanical work in the University summer school. Dr. Julia W. Snow and Mr. Pond will do work for the U. S. Fish Commission at Put-in-Bay.

THE PLANS of the botanical staff of the University of Iowa for the summer are as follows: Professor B. Shimek will be engaged in special studies of forestry problems in Iowa, under direction of the U. S. Department of Agriculture; Mr. T. E. Savage will probably complete his studies of the Mosses and Hepaticæ of the state; Mr. P. C. Myers will complete a photographic record of the diatoms of Iowa, recent and fossil; and Professor T. H. Macbride will probably spend the summer in the Grand Cañon of the Colorado, studying the forest problems there for report to the United States Department of Agriculture, and incidentally picking up the fungi of the region.

THE RHODE ISLAND College of Agriculture and Mechanic Arts, with the cooperation of Hon. Thomas B. Stockwell, state commissioner of public schools, and Dr. Horace S. Tarbell, superintendent of schools in Providence proposes to open a summer school for nature study at Kingston, R. I., from July 5 to 19, 1899. A general summer school is not contemplated, and the work offered by the various departments constitutes a single course dealing solely with local phenomena in their adaptability to the teaching of nature study. The distinctive feature will be the study of living nature rather than post-mortem biology. Plants will be studied in the field, supplemented by laboratory work when necessary to a clear understanding of the subject treated. No attempt will be made to determine species, but it is proposed to treat the following topics: relation of plants to each other, relation of plants to animals, adaptations for cross pollination, and adaptations for the distribution of seeds and fruits. There will be no entrance examination, and no special requirements for completion of the course.

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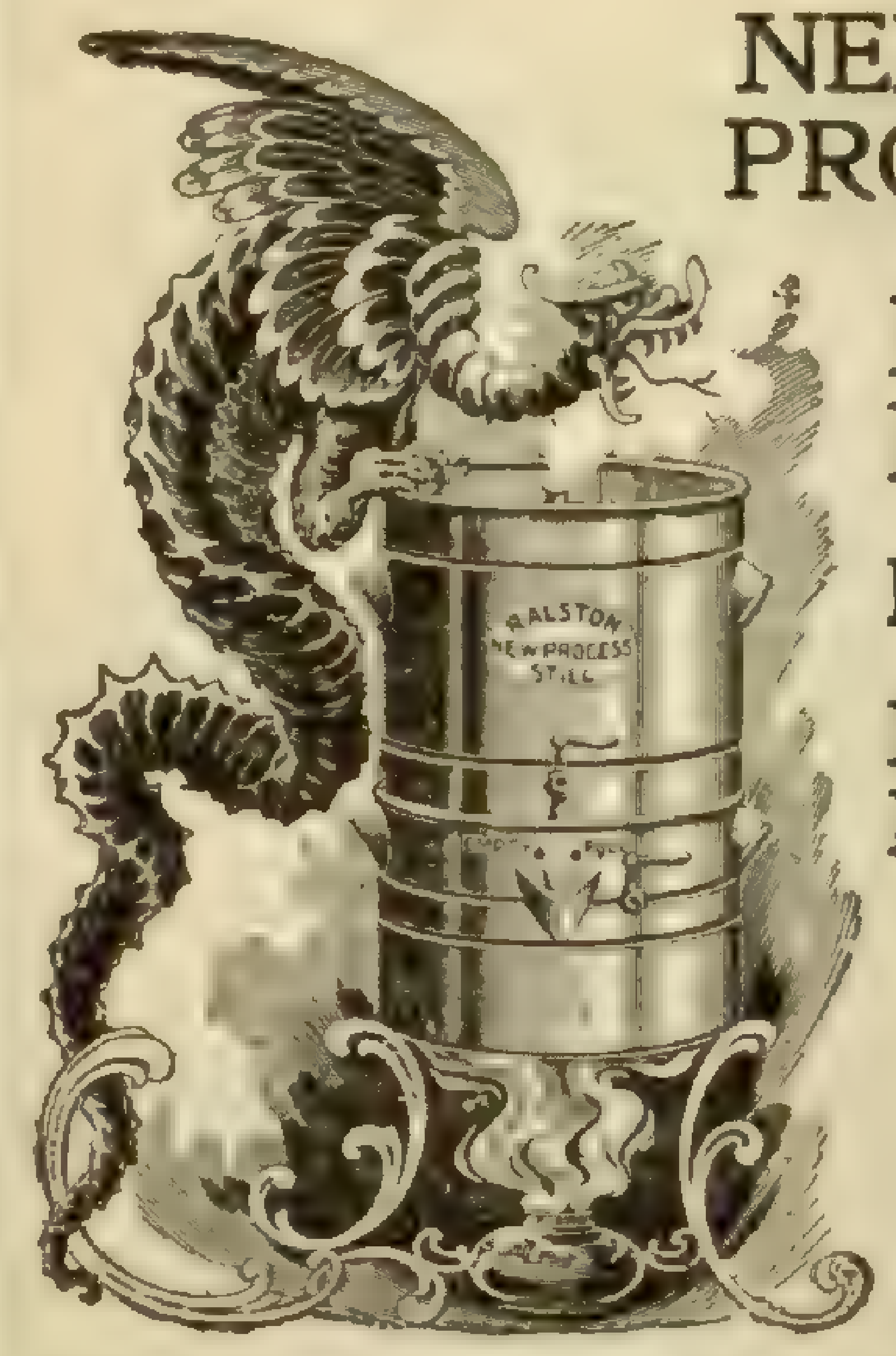

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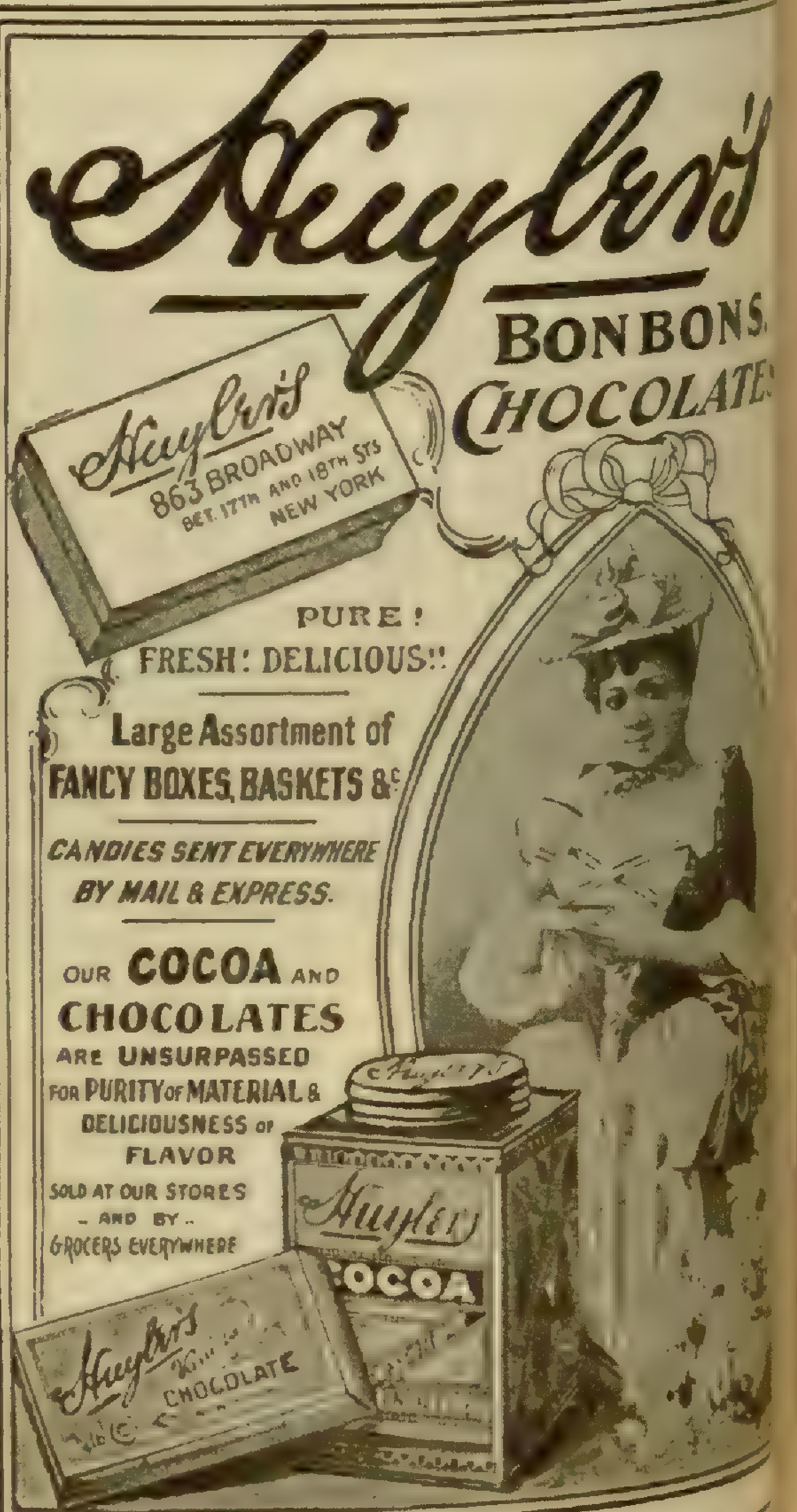

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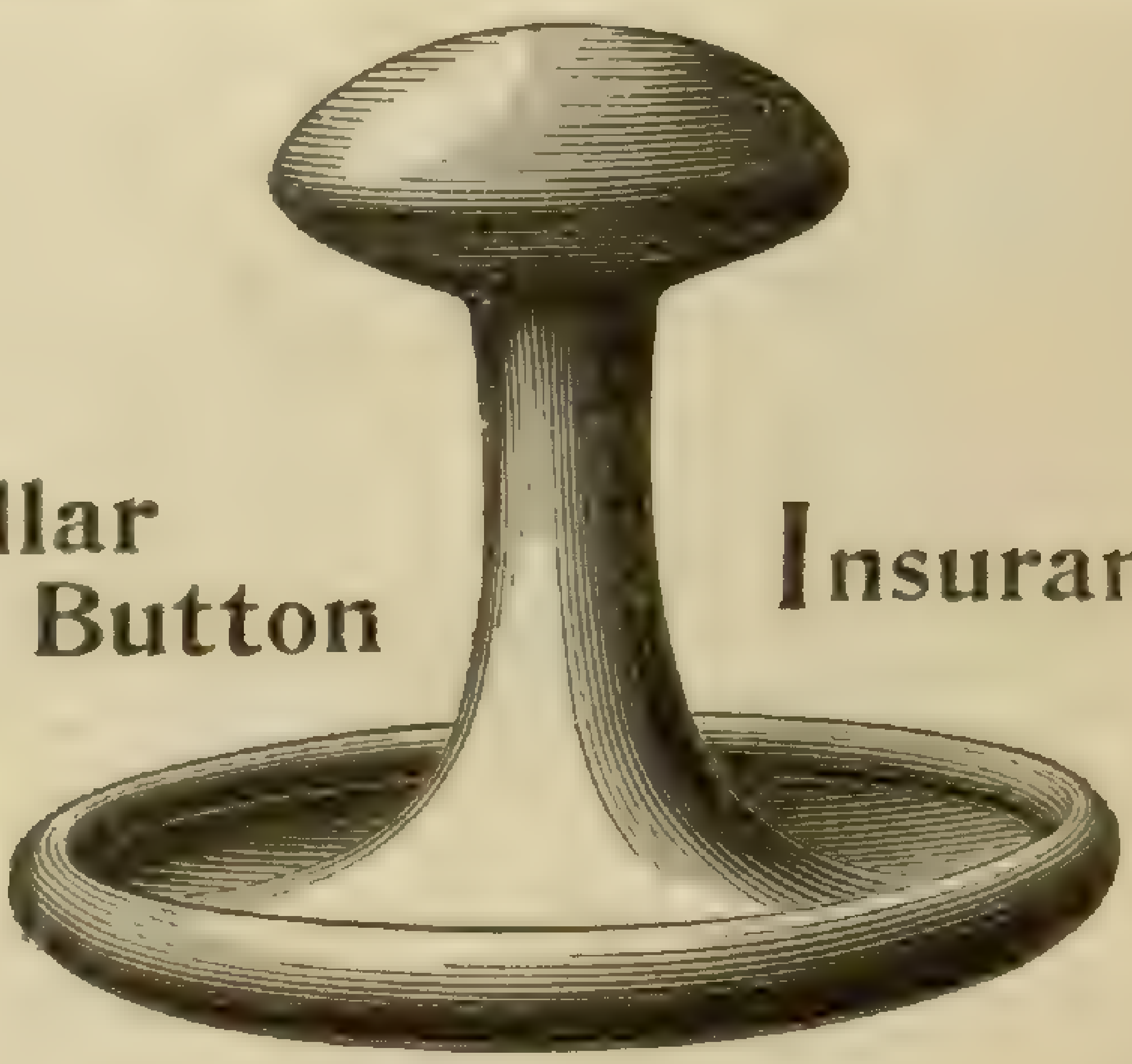

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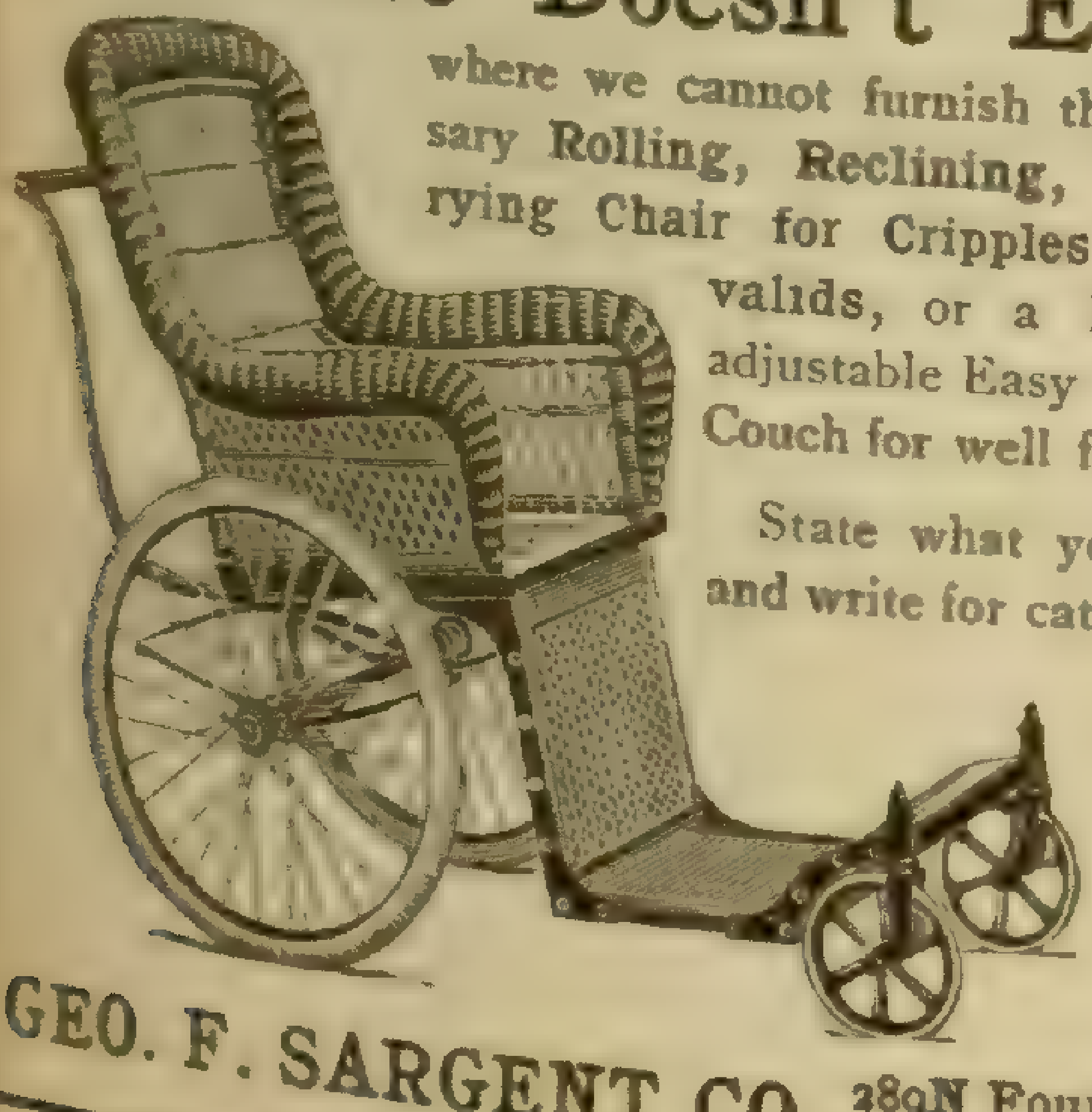

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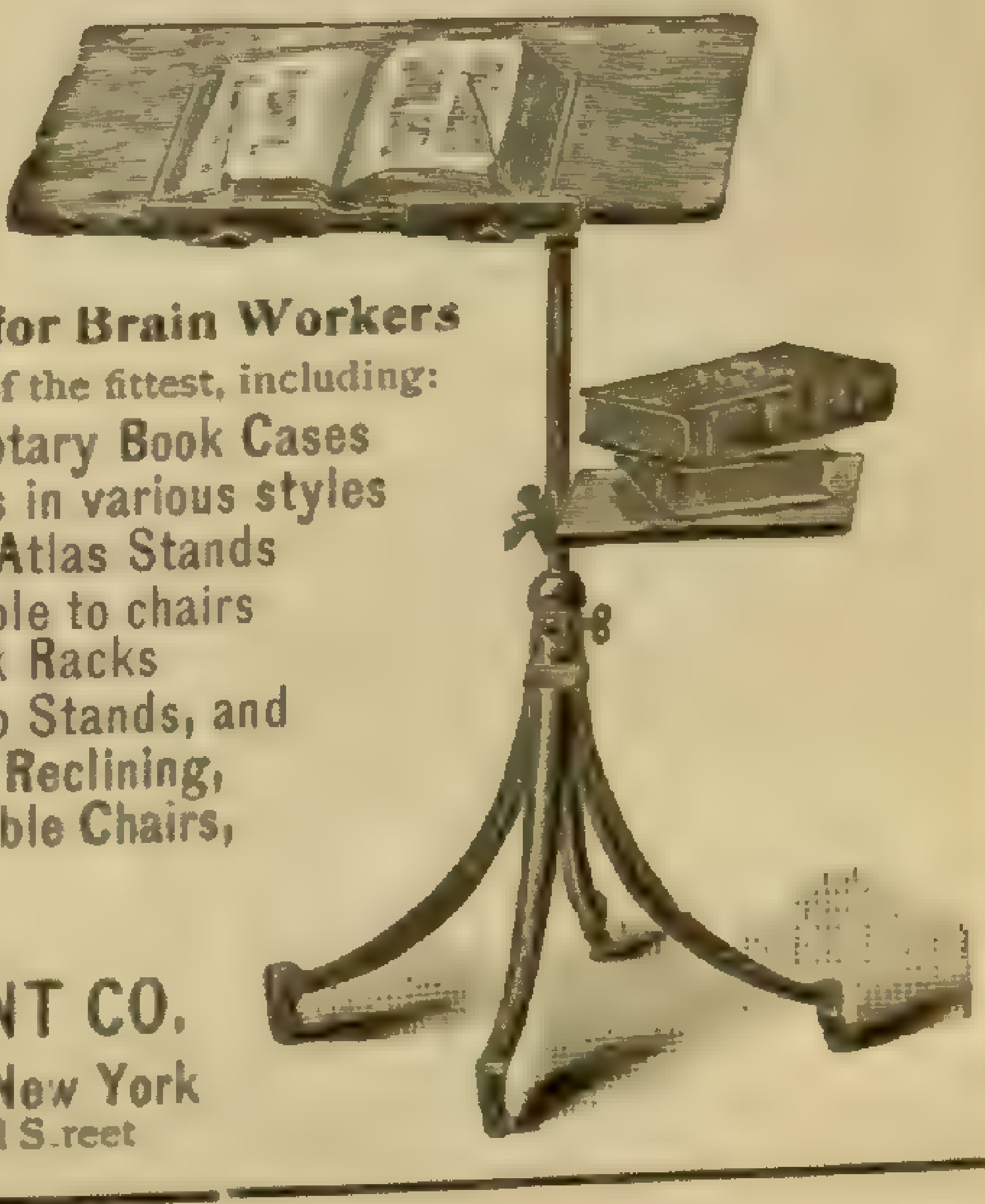

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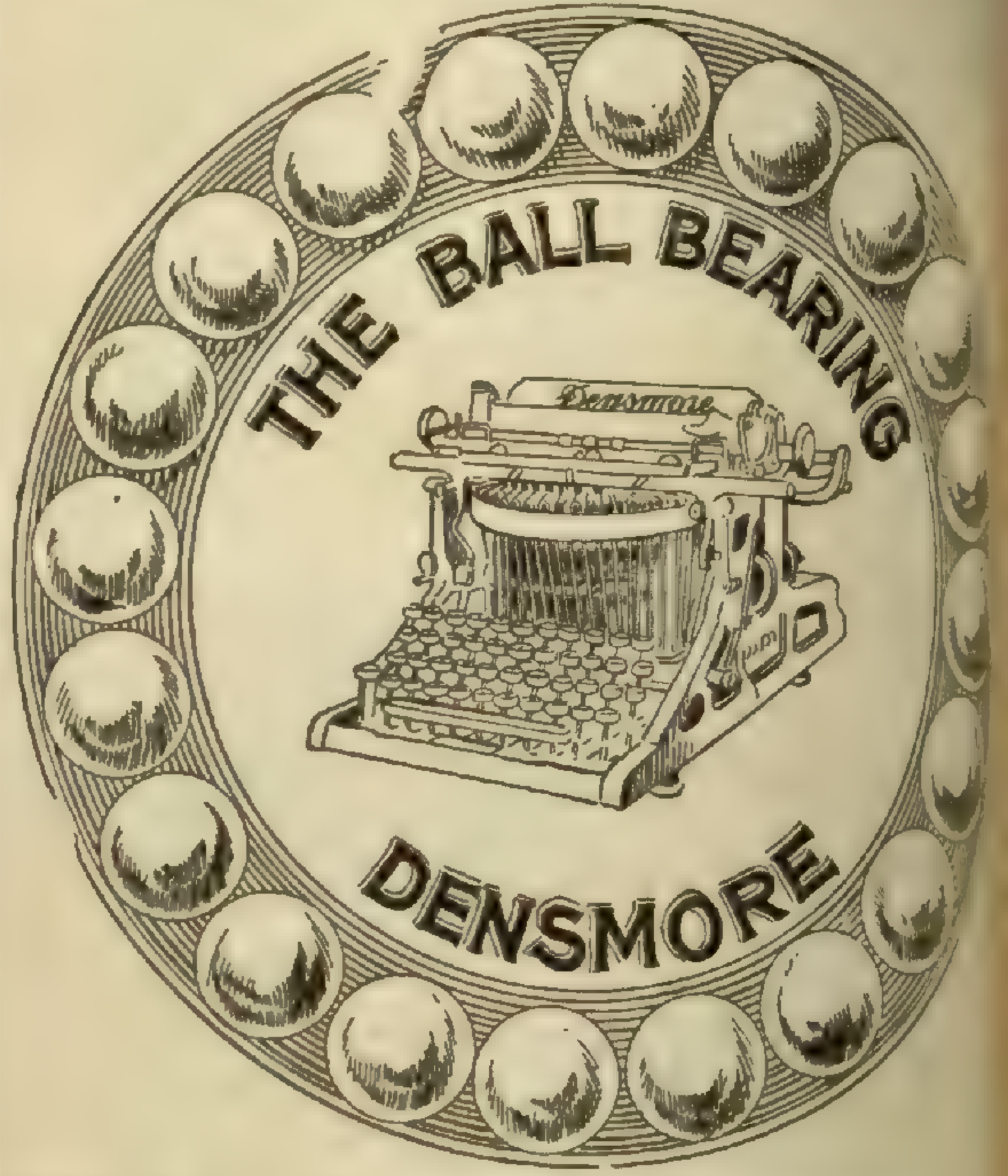

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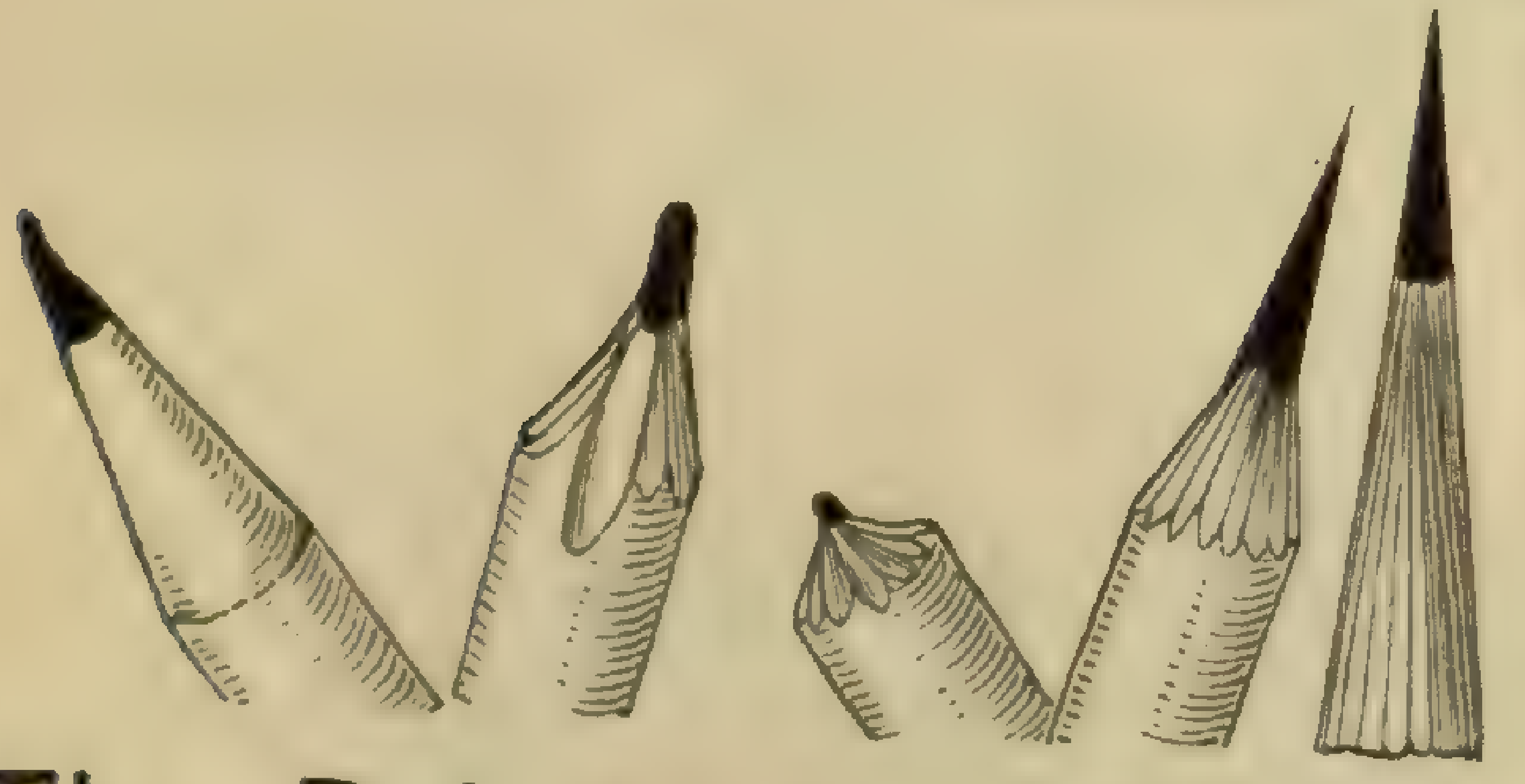

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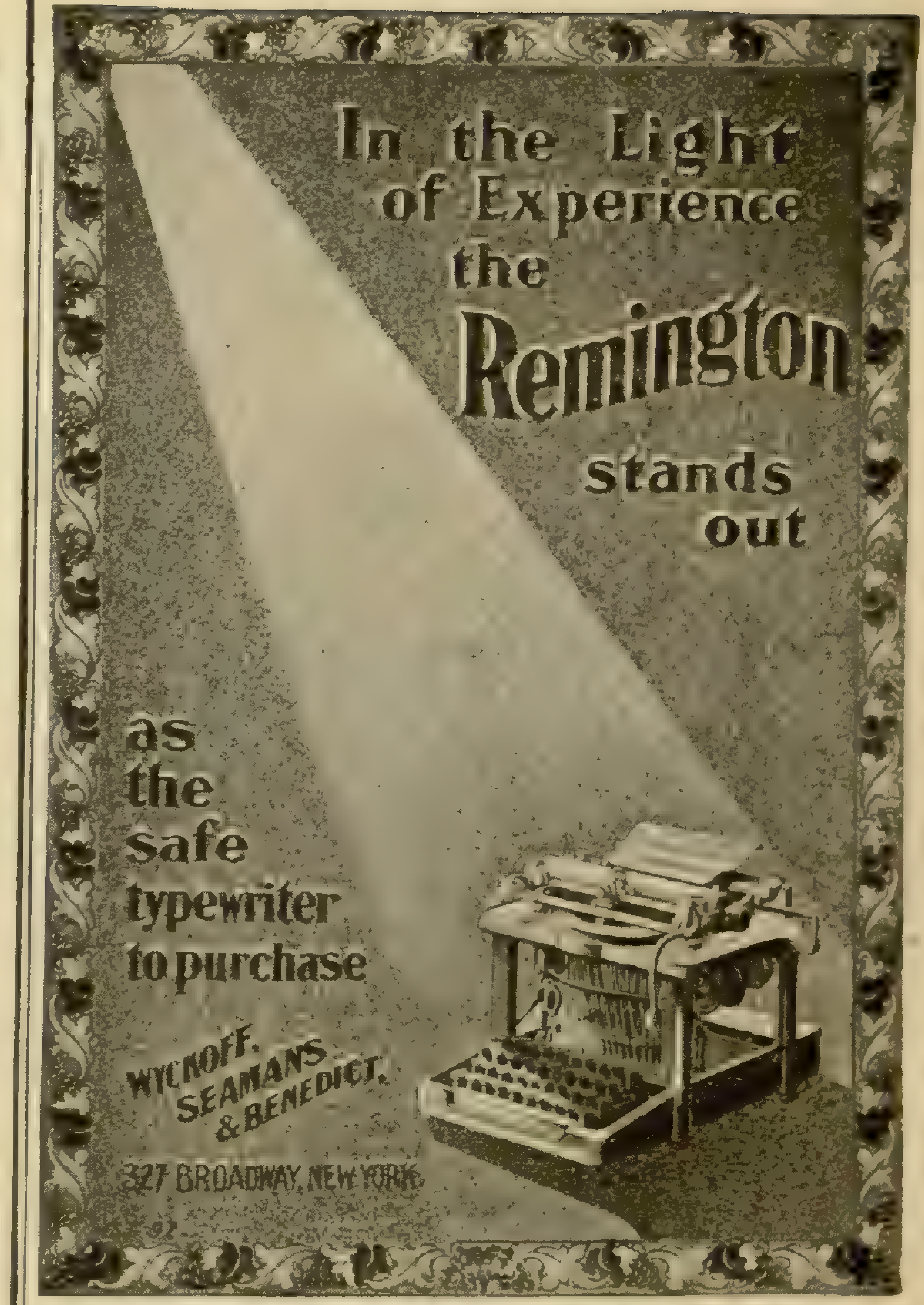

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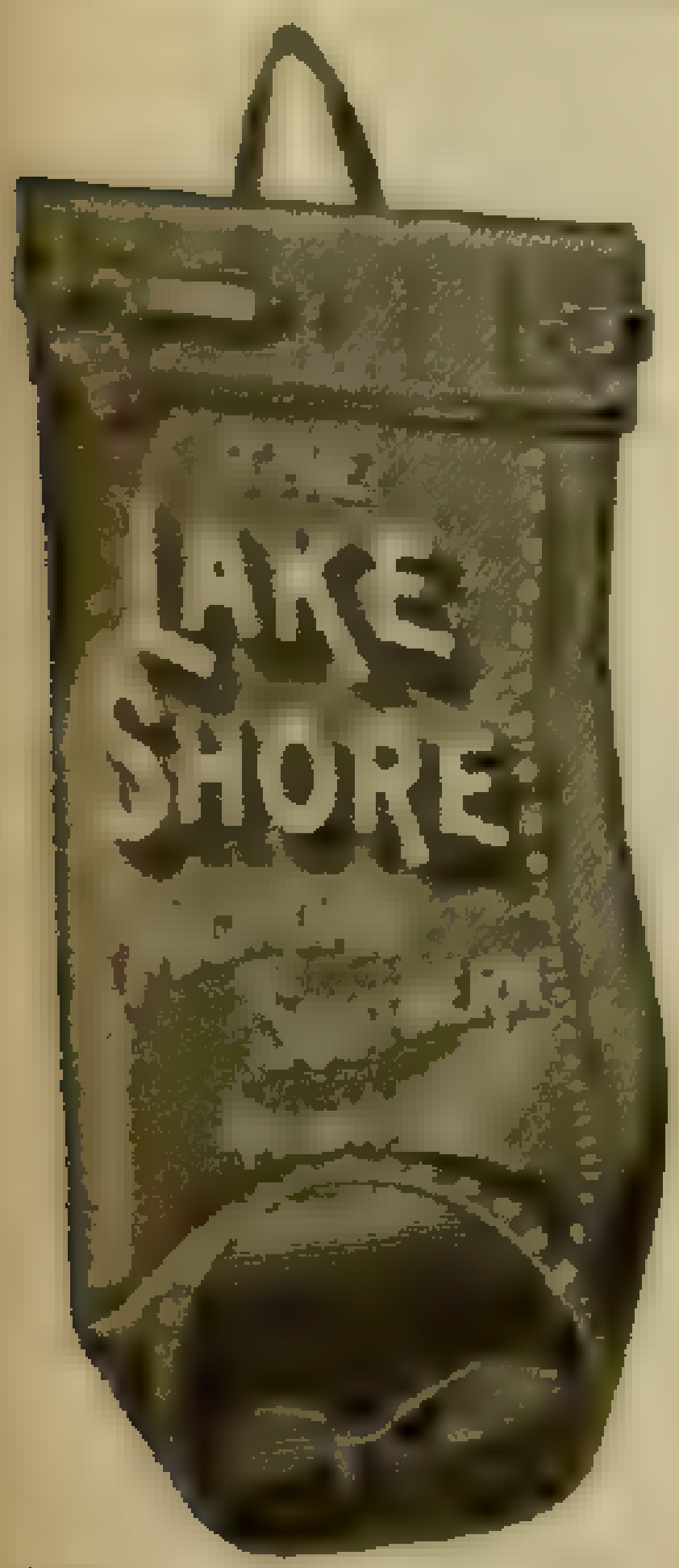

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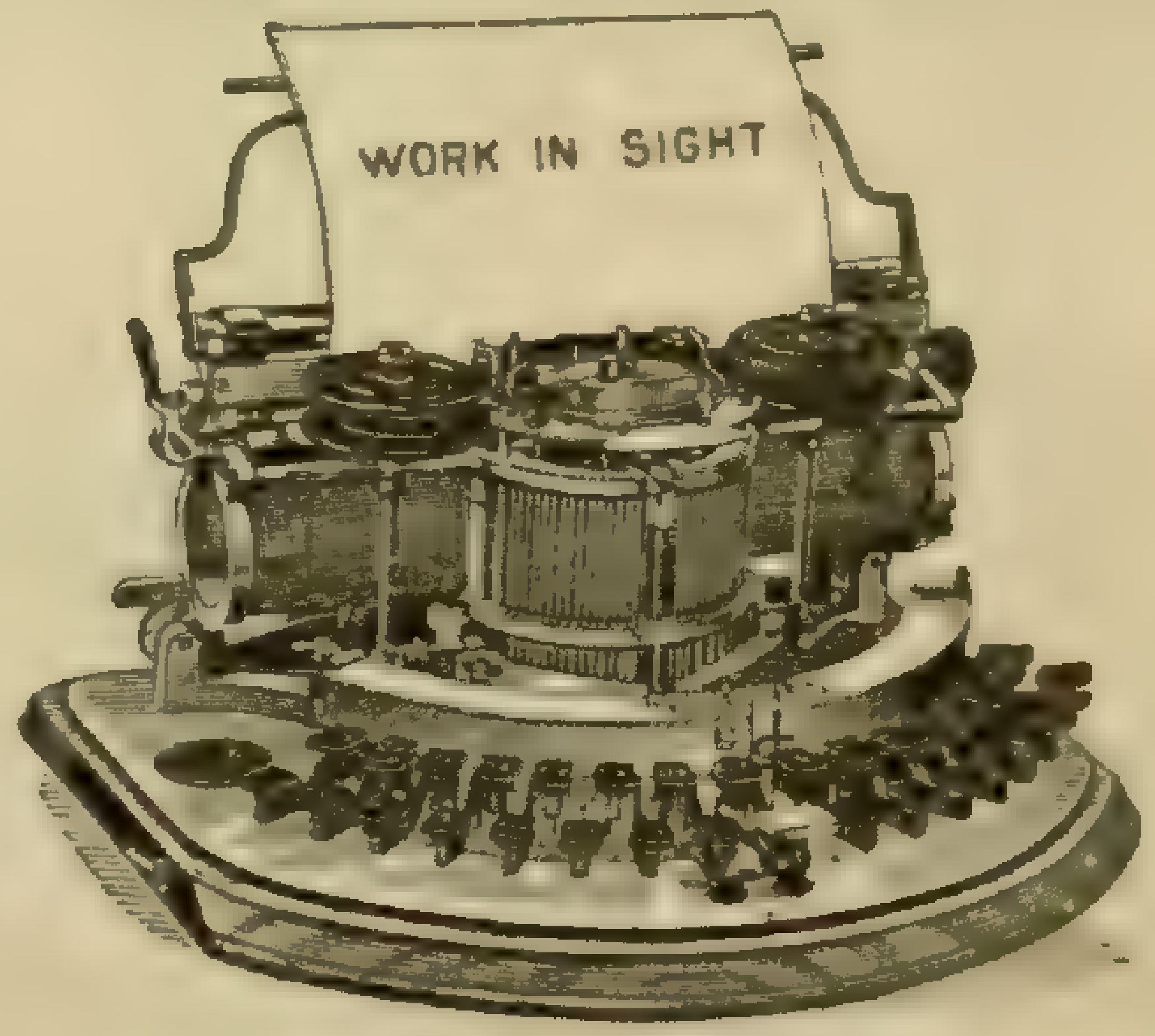

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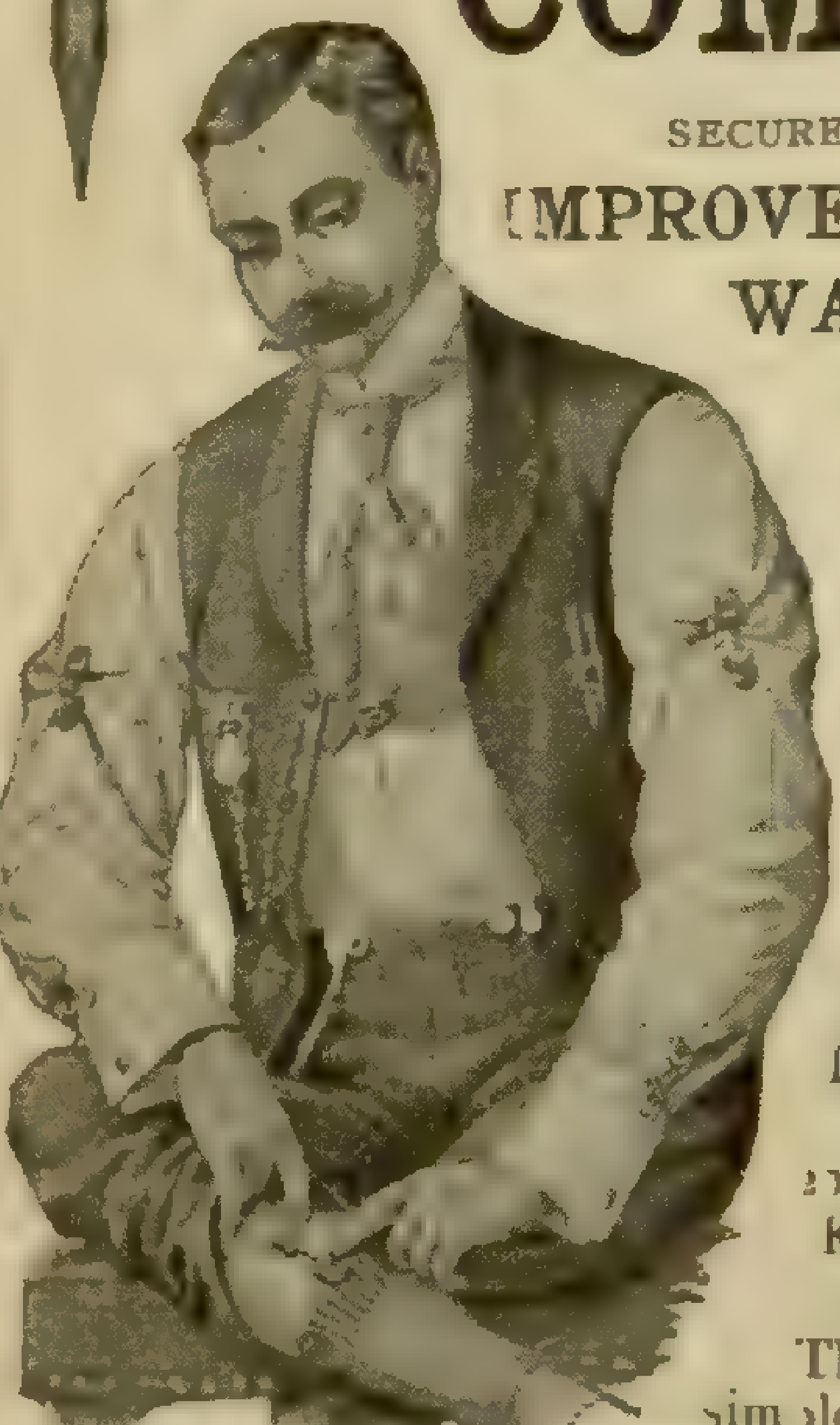

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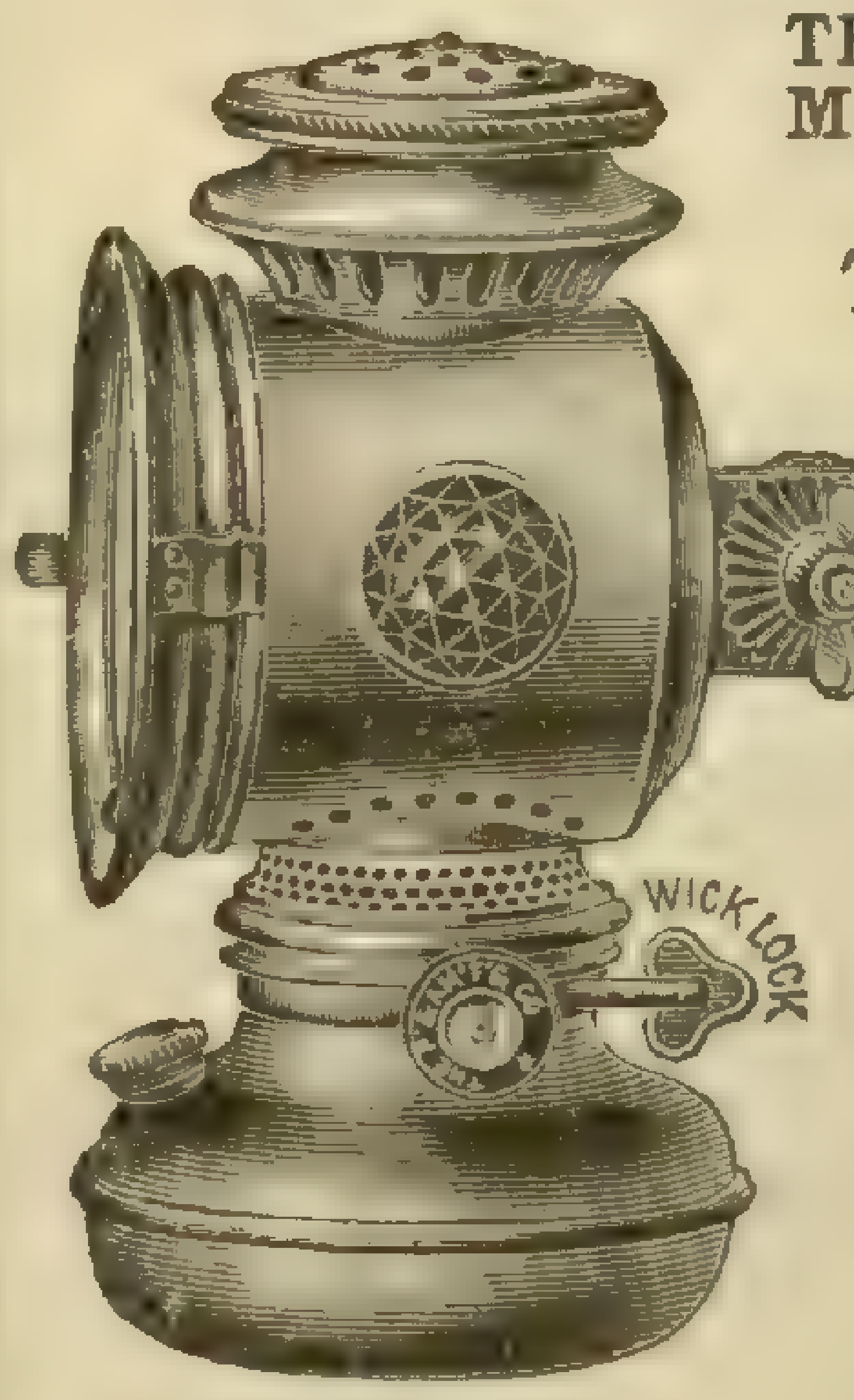

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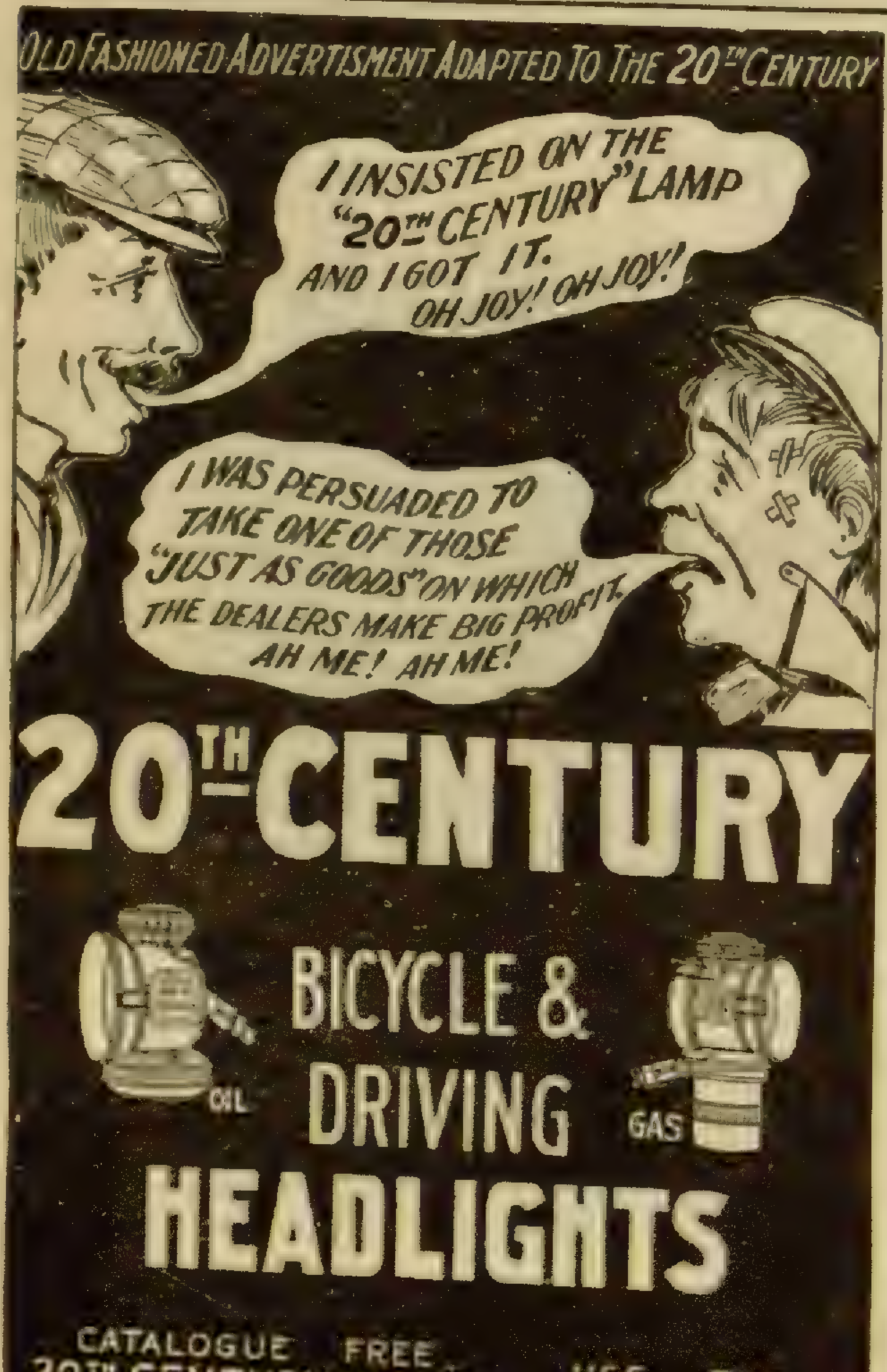
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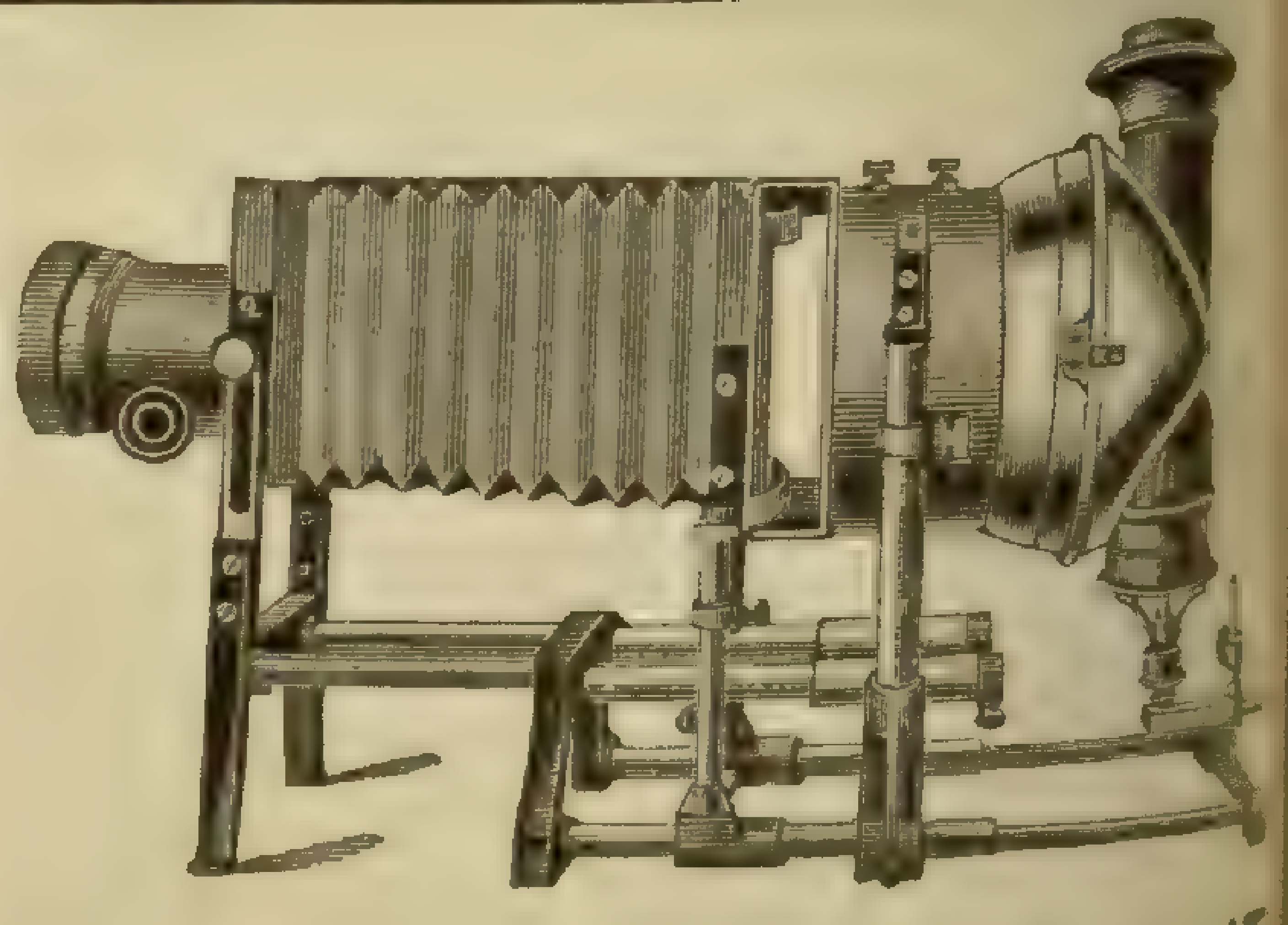

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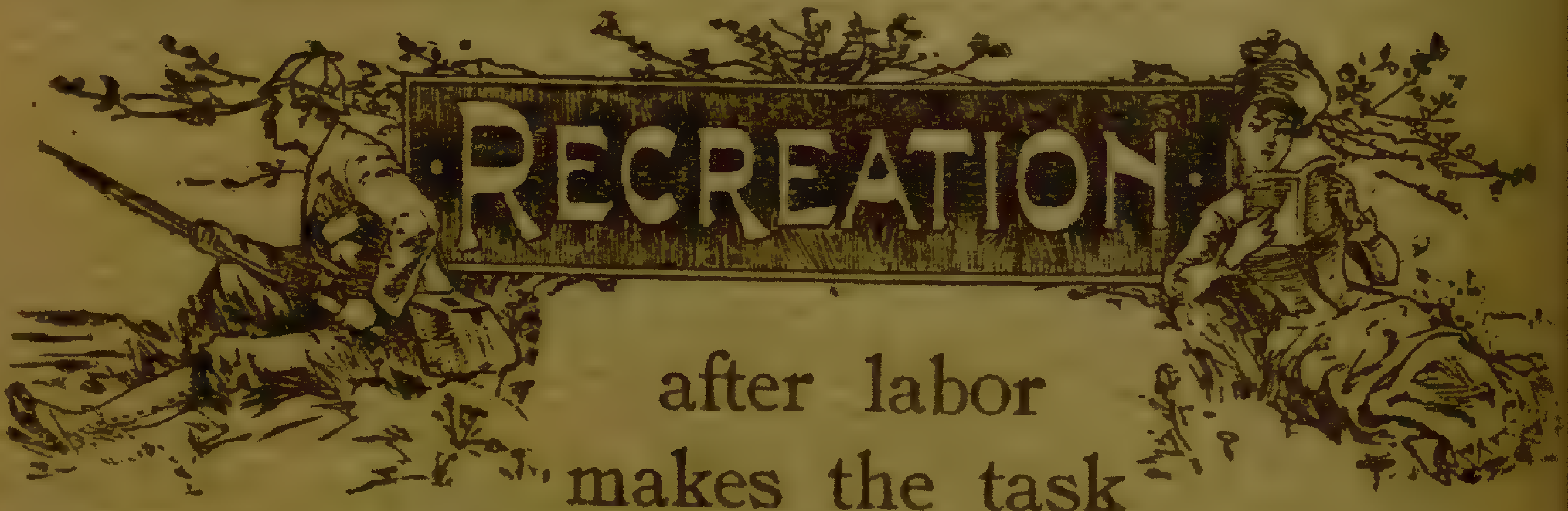
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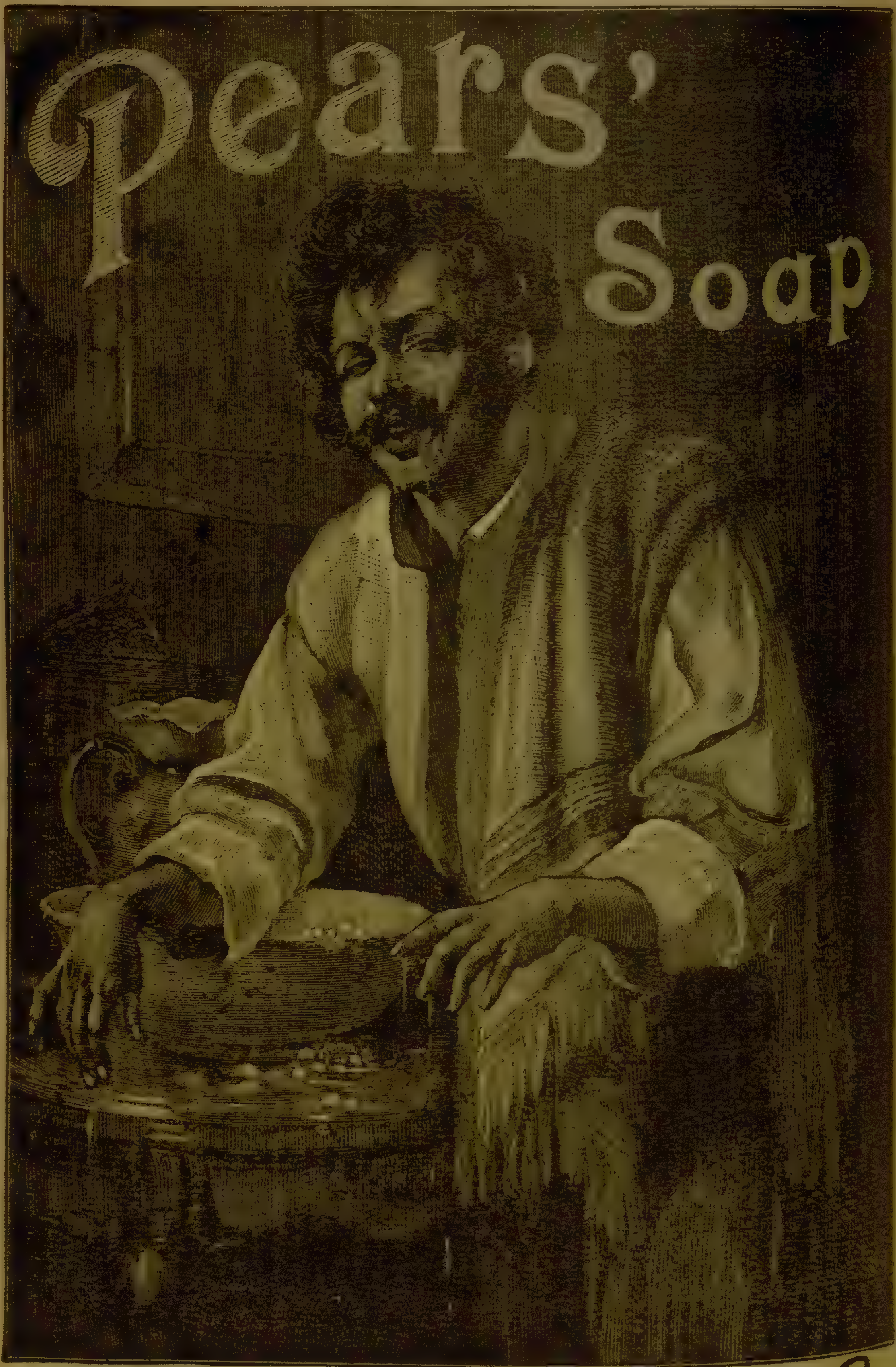

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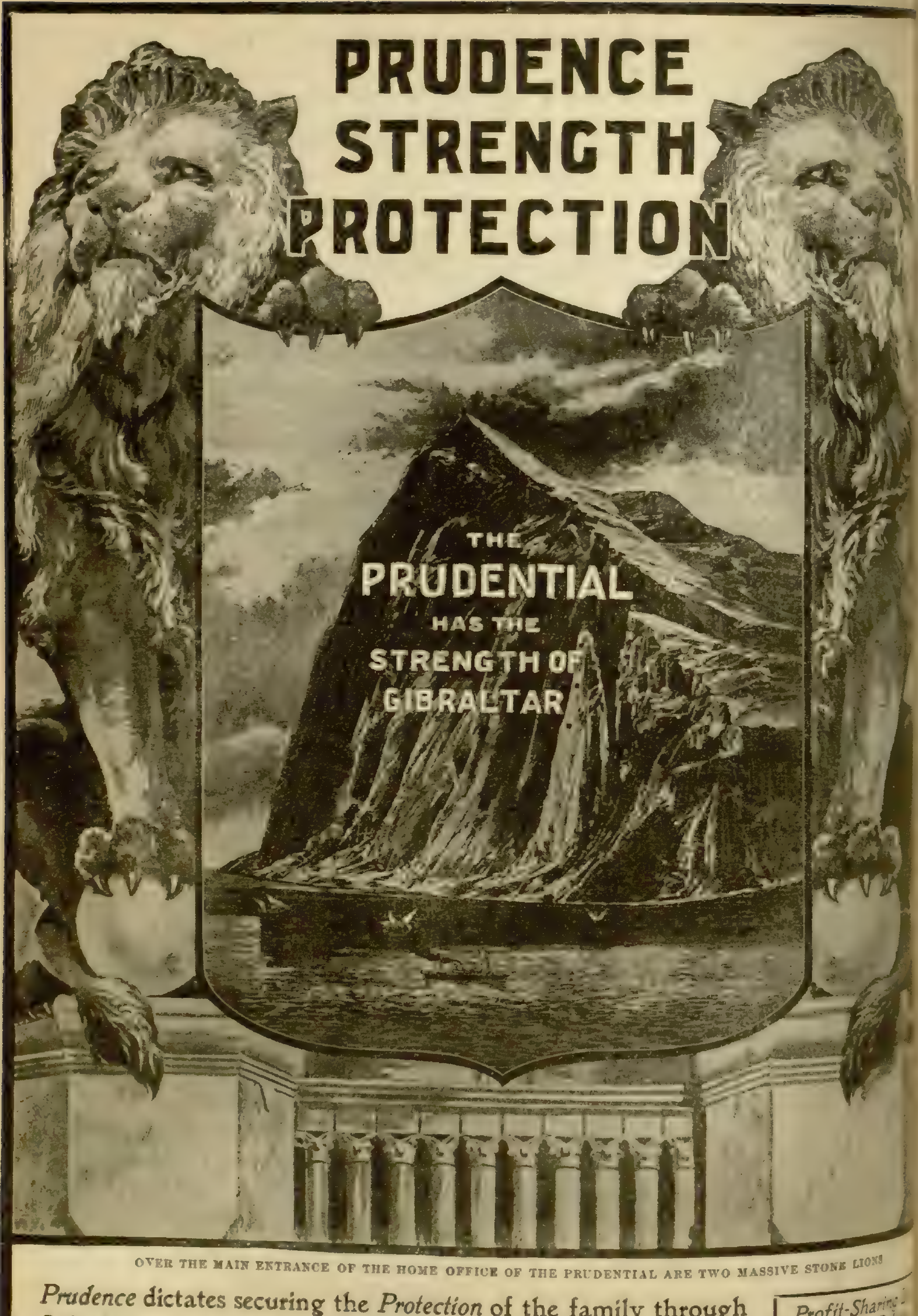
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BOTANICAL GAZETTE

AUGUST 1899

THE DEVELOPMENT OF THE MICROSPORANGIA AND MICROSPORES OF HEMEROCALLIS FULVA.[1]

EDWARD L. FULLMER.

(WITH PLATES VII–VIII).

ALTHOUGH *Hemerocallis fulva* has been a frequent subject of study, the results reported in reference to the phenomena of karyokinesis and spore formation do not seem to agree. In the *Cytologische Studien*,[2] this plant is described with multipolar spindles in the reproductive cells during the early division stages, no centrosomes being found in such cells. The division of the reproductive cells of the higher plants studied, according to the results of the several contributors to the *Studien*, is found to differ in both of these respects from the cells of the lower plants as well as from those of animals.

The spore-mother-cells of Hemerocallis are not very favorable for cytological study, the chromosomes being small and the spindles difficult to differentiate on account of a band of dark staining material found close around each nucleus; nor are the spindle threads so thick as those usually found. The dark band may be only an artifact, caused by the chromic acid of the killing solution. The material for this study was killed in chromacetic acid, and after being imbedded in paraffin was sectioned 12 and 14μ thick. The stains used were iron-alum-hæmatoxylin;

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[1] Contribution from the botanical laboratory of Ohio State University.

[2] Jahrbücher für wiss. Bot. 30: 1–268. *pl. 18.* 1897.

Flemming's triple stain; a combination of anilin-safranin with iron-alum-hæmatoxylin; and anilin-safranin followed by picro-nigrosin. The last named gave the best results in staining the incipient spindles.

DEVELOPMENT OF THE MICROSPORANGIA.

A cross section of a very young stamen, at the point where the microsporangia are to be formed, shows merely a rectangular area which consists of epidermal and general tissue cells. Three or four hypodermal cells of each sporangium become differentiated as archesporial cells. These divide by periclinal division giving rise to the primary sporogenous cells and the primary tapetal layer. The cells of the primary sporogenous tissue multiply rapidly, forming only sporogenous cells. The division of these cells is practically complete when the primary tapetal layer begins to divide. While the sporogenous cells are enlarging the division of the primary tapetal layer takes place, forming a wall layer and an inner layer. The inner layer divides once, forming an intermediate or middle layer, and the layer which develops into the peripheral part of the tapetum, the axial part being derived from the adjacent general tissue.

DIVISION OF THE MICROSPORE MOTHER CELL.

A typical spore mother cell when division begins (*fig. 1* shows a large nucleus in the center, with radiations in the cytoplasm extending from the nucleus outward toward the cell wall. There is usually a layer of deeper staining cytoplasm next the nucleus, and one or more large nucleoli just within the nuclear membrane. The young spindle is bipolar from its first appearance, each pole being dome-shaped for a time (*figs. 2, 10*). The spindle encloses the nucleus through which the chromosomes are scattered, and the layer of deep staining cytoplasm having moved outward from the nucleus spindle lies wholly within it. The radiations above described appear to be of the same nature and to have the same function as those described in animal cells such as fish eggs. If they are consumed in spindle formation they must be drawn entirely within the dark layer before the nuclear membrane has disappeared.

A careful study was made of preparations containing cells in various stages of division, many of which showed distinctly dome-shaped bipolar spindles just after the nuclear membrane had disappeared. Occasionally a nucleus was observed which seemed to have radiations extending outward, but these were never sufficiently distinct to be considered as forming a multipolar spindle. The spindle was not differentiated by the stains in any case until after the nuclear membrane had almost disappeared, as the dark band obscured the poles. At first these alone would be visible, since the remainder of the spindle could scarcely be distinguished from the nuclear membrane because of their close proximity; the central part of the spindle perhaps being in contact with the nuclear membrane. The spindle fibers at this stage are delicate, the spindle being in process of formation. The fact that the nuclear membrane disappears while the spindle is forming lends support to the theory that the material of the nuclear membrane is consumed in spindle formation.

As the poles separate, the spindle gradually becomes pointed and seems to grow somewhat narrower in the middle portion, so that it is not as wide as was the original nucleus (*figs 1–7, 10–12*). The chromosomes are drawn into the equatorial plane (*figs. 8, 14*) soon after the spindle becomes elongated to definite points. Small deep-staining bodies which have the appearance of centrosomes are often found at the poles in the various stages of both the first and second divisions (*figs. 3, 7, 9, 13*).

In the second division of the spore-mother-cell the two nuclei may divide successively (*fig. 22*), but almost always simultaneously (*figs. 14, 16*), the spindles usually being parallel to one another (*fig. 15*), but occasionally obliquely placed (*fig. 16*), and often persisting even after division is complete (*fig. 17*). The phenomena of the first and second divisions are the same, except that in the latter the nuclei are smaller and the dark band is not so conspicuous.

Four microspores (*figs. 18, 19*) are usually produced from each spore-mother-cell, but occasionally five, six, and even eight are formed (*figs. 20–27*). Miss Lyon[3] also found this peculiarity in *Euphorbia corollata*, which in the case of Hemerocallis has been known for some time. The extra nuclei were first described as being produced by one or more of the four tetrad nuclei dividing by karyokinesis. Recently, however, they have been described by Juel[4] as being formed from chromosomes which became isolated in one of the divisions of the spore-mother-cell. Many tetrads having supernumerary nuclei were examined, only a few of which seemed to show definitely how the extra number of nuclei might have arisen. In all cases where the origin was indicated by spindles or otherwise they seemed to have been produced by a subsequent karyokinesis. The extra nuclei in *figs. 20* and *21* may be a result either of the indirect division of one of the cells of the tetrad, or of the threefold division of one of the nuclei at the second division. The division of one nucleus into three or more is quite common in animal cells in case of pathological tissue. Wilson,[5] in speaking of cases of pathological mitosis, says:

> The abnormal forms of mitoses are arranged by Hanseman in two general groups, as follows: (1) *asymmetrical mitoses*, in which the chromosomes are unequally distributed to the daughter cells, and (2) *multipolar mitoses*, in which the number of centrosomes is more than two, and more than one spindle is formed. . . . . Lustig and Galeotti ('93) showed that the unequal distribution of chromatin is correlated with and probably caused by a corresponding inequality in the centrosomes, which causes an asymmetrical development of the amphiaster.

The same author refers to the discovery of Galeotti that asymmetrical mitoses may be artificially produced in the epithelial cells of salamanders by treatment with various drugs. Guignard[6] finds very irregular and also multipolar spindles in the spore-mother-cells of *Nymphæa alba* and *Limodorum abortivum*, and also in the tapetal cells of *Magnolia Yulan*. A number of spindles from cells of the above-named plants and also from the cells of *Nuphar luteum*, having well defined centrospheres and radiations around the poles, are shown.

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[3] A contribution to the life history of *Euphorbia corollata*. BOT. GAZ. **25**: 418–
1898.

[4] Jahrbücher für wiss. Bot. **30**: 205–226. 1897.

[5] The cell in development and inheritance, 67–68. 1896.

[6] Ann. Sci. Nat. Bot. VIII. **6**: 177–220, *pl. 9–11*. 1898.

If the fifth nucleus in *figs. 20* and *21* was formed by the division of one of the tetrad nuclei, the division was probably normal. In this case the presence of spindles suggesting tripolar mitosis would be explained by the persistence of spindle structures as in *fig. 17*. *Fig. 22* is similar to *figs. 20* and *21*, except that one of the cells resulting from the first division of the spore-mother-cell did not divide. *Fig. 23* may have arisen by any one of the processes described under *figs. 20* and *21*, but since there is no spindle connecting either of the two smaller nuclei with one of the larger, they may have arisen from a chromosome which was isolated in the first division of the spore-mother-cell, forming a nucleus and afterward dividing. In this case but one of the cells resulting from the first division could have divided, as there are only three other nuclei present. However, the three are nearly uniform in size, and all have the general appearance of tetrad nuclei, and not the appearance of a nucleus formed in the first division, as the elongated nucleus in *fig. 22*, which has the usual shape of such nuclei.

Fig. 24 shows four nuclei, two of which are dividing by karyokinesis. The appearance and position of the cell walls, as well as the unequal size of the nuclei, suggests that the two nuclei resulting from the first division of the spore-mother-cell were of unequal size. By the subsequent division of the nuclei thus formed two pairs of nuclei resulted; the larger pair of which are again dividing by karyokinesis. This figure may also be explained by supposing the two smaller nuclei to have been produced from an isolated chromosome. In this case the spore-mother-cell could have divided but once, and is now dividing a second time. One of the five nuclei in *fig. 25* is dividing. The source of this nucleus cannot be determined with certainty; but it may have been formed either by the indirect division or by the fragmentation of one of the tetrad nuclei, more probably the smallest one The fact that several, perhaps the normal number of chromosomes are present opposes the view that it arose from an isolated chromosome. Six nuclei are found in *fig. 26*, the origin of four being shown by spindles. The two extra nuclei are quite small, containing but little chromatin, and may have been produced by isolated chromosomes as above described. However, if they were produced in this manner the smallest of the four nuclei must have arisen by pathological mitosis by an unequal distribution of chromatin, for it is very small and contains but little chromatin. *Figs. 27* and *28* show tetrads with supernumerary cells. The cells of such tetrads are seldom of equal size, one or more of the cells usually containing a small nucleus.

THE DEVELOPMENT OF THE MALE GAMETOPHYTE.

The microspores soon after their separation and rounding off acquire the general shape and characteristic markings of the mature pollen grain, gradually becoming very much enlarged (*figs. 28, 30*). A single elongated nucleus, which seems to have a small amount of chromatin, is found in the center of the spore until growth is far advanced; after which the division into generative and tube nuclei occurs (*fig. 30*). Until recently it was thought that the tube nucleus never divides, but Chamberlain[7] found many cases of such division in *Lilium tigrinum* and *L. auratum*. Smith[8] found the same thing to be of frequent occurrence in *Eichhornia crassipes*. Hemerocallis also shows this peculiarity (*figs. 31–35*), many pollen grains having two, and a few having three, four, or even six tube nuclei. These were formed by direct division in all the cases that I observed, as were those reported by Chamberlain. *Fig. 33* shows a very irregular tube nucleus.

If the tube nucleus is the homologue of the cover cell of the antheridium of Marsilia, and represents the wall of an antheridium, it might sometimes divide through reversion to its more

[7] Bot. Gaz. **23**: 423–430. 1897.

[8] Bot. Gaz. **25**: 324–337. 1898.

primitive condition. Since extra tube nuclei are produced only by fragmentation, so far as known, such division may represent a pathological condition. It is possible that direct division in the higher plants never represents anything more.

SUMMARY.

1. Three or four hypodermal cells of each sporangium become differentiated as the archesporial cells. The wall of a sporangium consists of three layers exclusive of the epidermis. The tapetum is a physiological rather than a morphological structure, the peripheral part being organized from the wall layers and the axial part from the general tissue.

2. The spindle appears bipolar from its first appearance, being dome-shaped in the early stages. No trace of multipolar spindles was observed. The spindles often persist for a considerable time after division is complete. Bodies having the appearance of centrosomes are frequently seen at the poles.

3. The origin of the supernumerary microspores was not absolutely determined. In many cases where their origin was indicated by spindles or otherwise they appeared to arise by the indirect division of one of the tetrad nuclei.

4. The tube nucleus frequently divides by direct division, forming sometimes as many as six or eight nuclei.

In conclusion I wish to express my thanks to Dr. W. A. Kellerman and Mr. J. H. Schaffner for valuable assistance and criticism.

COLUMBUS, O.

EXPLANATION OF PLATES VII AND VIII.

The figures were drawn with a camera lucida and are reduced to three eighths their original size. *Fig. 32* was drawn with a Bausch and Lomb $\frac{1}{12}$ objective and a Leitz no. 2 ocular (× 1200); *figs. 3, 4, 9, 10, 13, 15, 21, 22, 23* with a Leitz $\frac{1}{16}$ objective and a Leitz no. 4 ocular (× 1600); all others with a Bausch and Lomb $\frac{1}{12}$ objective and a Zeiss no. 6 ocular (× 1600).

PLATE VII.

FIG. 1. Microspore mother-cell in very early stage of first division, showing dark band around nucleus and cytoplasmic radiations extending outward to the cell wall.

FIG. 2. Loose mother skein stage, showing a dome-shaped bipolar spindle which is situated entirely within the dark band.

FIGS. 3–5. Successive division stages, showing centrosome-like bodies at poles of dome-shaped spindles.

FIG. 6. Same as above, but having no bodies visible at the poles.

FIG. 7. Mother star stage; spindle pointed, having centrosome-like bodies, and with radiations at lower pole.

FIG. 8. Metakinesis stage; microspores separated.

FIG. 9. Near close of metakinesis; centrosome-like bodies and radiations present.

FIG. 10. Early stage of second division, showing dome-shaped spindles with centrosome-like bodies; dark band not so prominent as in first division.

FIG. 11. Showing one cell in a later stage of division than is the other.

FIGS. 12–14. Successive division stages.

FIG. 15. Loose daughter skein stage, showing an isolated chromosome; spindles parallel.

FIG. 16. Same as above, showing spindles lying obliquely to one another.

FIG. 17. Tetrad having nuclei variable in size and showing persisting spindles.

FIGS. 18–19. Later tetrad stages.

PLATE VIII.

FIGS. 20–21. Mother cells with five nuclei, three of which are connected by spindles, showing their common origin.

FIG. 22. Same as above, except that one nucleus did not divide after first division of spore-mother-cell.

FIG. 23. Five nuclei in mother-cell, two pairs of which are connected by spindles.

FIG. 24. Four nuclei in tetrad, two of which are dividing by karyokinesis.

FIG. 25. Mother-cell with five nuclei; one is dividing by karyokinesis.

FIG. 26. Mother-cell with six nuclei; the origin of two not shown.

FIGS. 27–28. Five- and six-celled mother cells.

FIG. 29. Microspore soon after separation.

FIG. 30. Normal mature pollen grain having one generative and one tube nucleus.

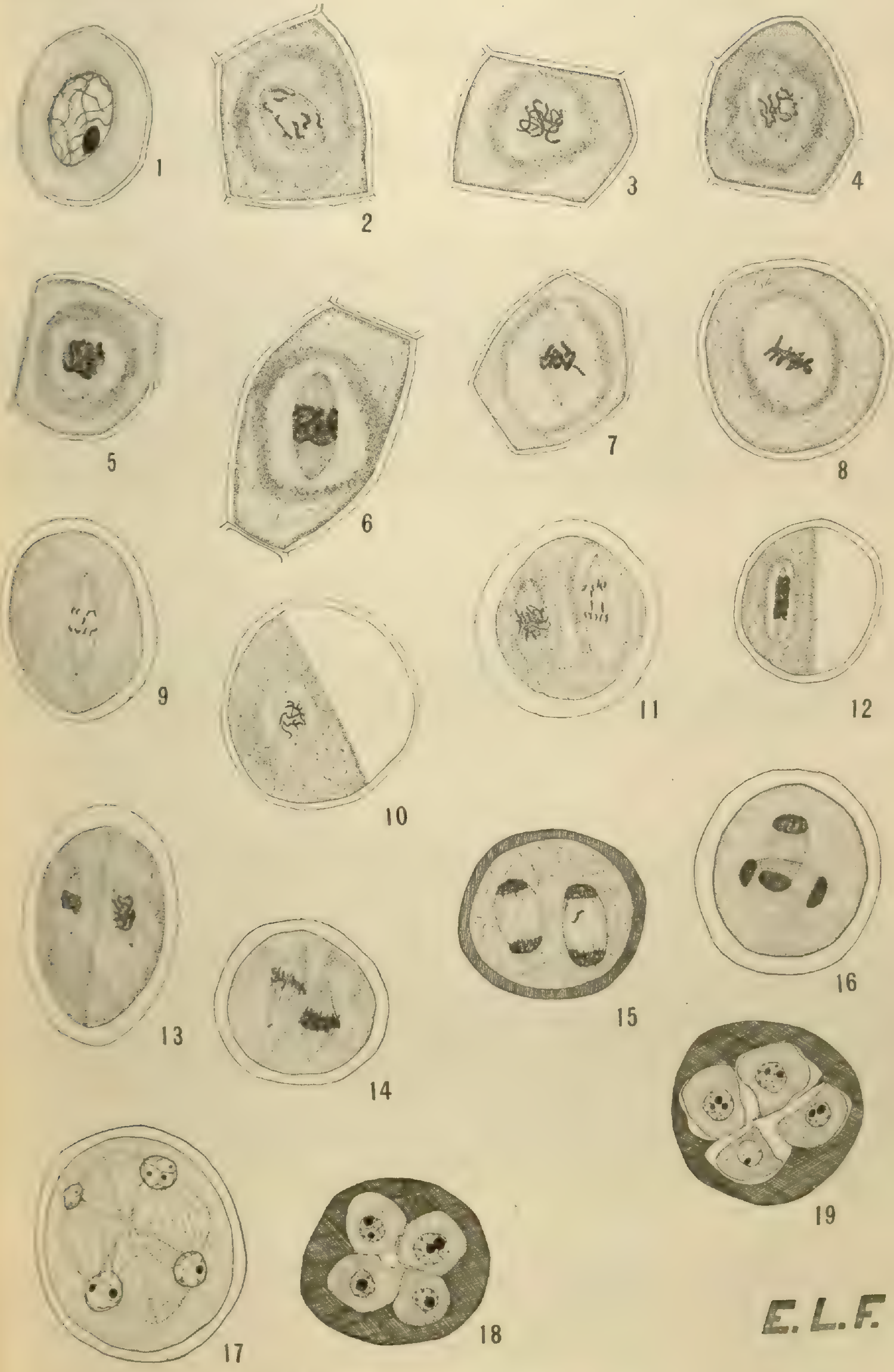
FIG. 31. Tube nucleus of pollen grain dividing by direct division.

FIGS. 32, 34. Pollen grains with one generative and two tube nuclei.

FIG. 33. Pollen grain with a very irregular tube nucleus.

FIG. 35. Pollen grain with three tube nuclei; generative nucleus not shown.

FIG. 36. Six tube nuclei in pollen grain.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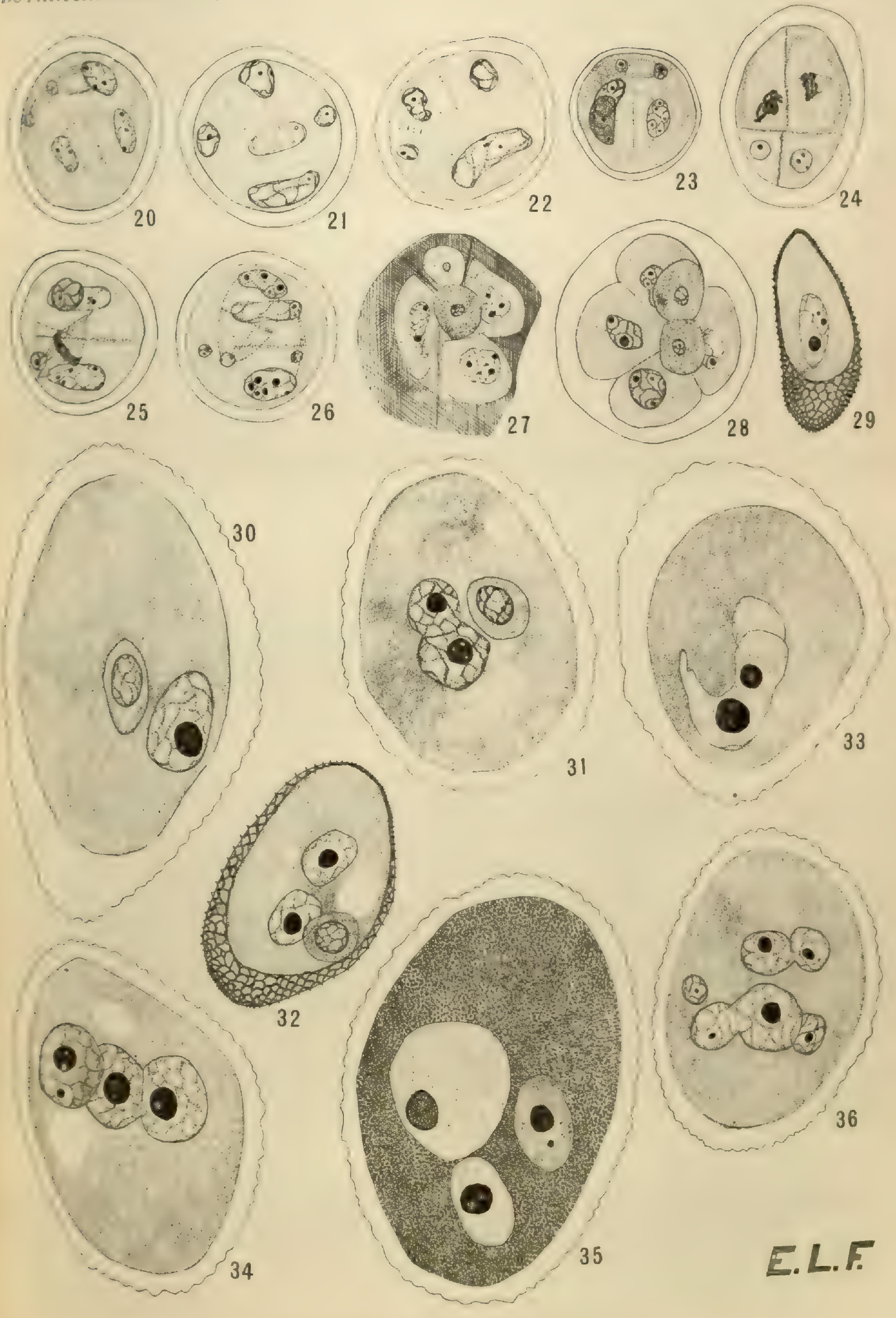
FULLMER on HEMEROCALLIS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FULLMER on HEMEROCALLIS

THE SPORE-MOTHER-CELL OF ANTHOCEROS.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XV.

BRADLEY MOORE DAVIS.

(WITH PLATES IX AND X)

ANYONE who has ever examined sections of the sporogonium of Anthoceros must have been impressed with the extreme beauty of the spore-mother-cells as they are exhibited in all stages of development throughout the length of this structure. The exceptional chloroplast and the various conditions illustrating the division of the cell-contents to form the spores are perhaps the most striking features presented. These led the writer to attempt a detailed examination in the hope that light might be thrown on certain problems that interest the student of plant cytology

The work of Farmer ('94 and '95) is the only contribution of a detailed nature upon the cytology of the Hepaticæ. He did not study Anthoceros but confined himself chiefly to certain thalloid Jungermanniaceæ and reported some very interesting and remarkable conditions. The present investigation does not agree with his accounts of the processes of nuclear division presented by such forms as Pallavacinia, Aneura, Fossombronia, and Pellia. However, at the outset the writer wishes to express no surprise at important differences between such divergent forms as Anthoceros and members of the Jungermanniaceæ. The researches of the past few years in cytology have certainly taught us to be wary of generalizing upon the conditions illustrated by particular cases.

This investigation deals with the single species *Anthoceros laevis* L., the material being collected at Woods Hole, Mass., where were conducted the preliminary studies and experiments on methods of fixation. The best results were obtained from specimens that had been fixed for twenty-four hours in weak Flemming solution of the following formula: 1 per cen chromic acid 25^{cc}, 1 per cent. glacial acetic acid 10^{cc}, 1 per cen osmic acid 10^{cc}, water 55^{cc}. Much material, however, was kill in other ways, sectioned and stained to determine as precisely a possiblė the merits of Flemming and the disadvantages of oth killing agents. The results of these experiments are brough together at the end of the paper and present some interestin data.

Sections were cut 5 and 7.5 μ thick from material embedd in paraffin and for the most part stained after Flemming's metho with safranin, gentian violet and orange G. Excellent resul were obtained with iron alum and haematoxylin employed according to the principles of Haidenhain. Acid fuchsin followed b methyl green or gentian violet presented interesting examples o differential staining, but the results were not as good as thos given by Flemming's triple stain.

At the outset it will be well to state that the number o chromosomes for *A. laevis* appears to be four and eight in th gametophyte and sporophyte respectively. The count in th gametophyte is based solely upon observations of many mitot figures in the cells of a developing antheridium, but it agree with the number presented in the nuclear figures in th spore-mother-cell. The number eight for the sporophytic generation was presented quite frequently and in different regio of that structure. Thus the archesporium just before the differentiation of the spore-mother-cells, the tissue below the archesporium, and the cells of the columella afforded many nucle figures in which a count could be made with reasonable accura

The attractiveness of the sporogonium of Anthoceros as subject for study lies largely in the fact that in one structu there may be present a very complete series of stages from th time when the spore-mother-cell is differentiated until the spore are fully ripe. It is possible from this fact to relate to eac other with great exactness the events that take place as th spore-mother-cell matures.

As is well known, the sporogonium of Anthoceros grows from a region of embryonic or meristematic tissue in the slightly constricted portion of the structure just above the foot. From this region there rises a central columella around which are arranged in the form of a hollow cylinder the cells destined to develop into spores and sterile cells (elaters). Recently published accounts of the structure and development of the sporogonium by Campbell (**'95**, pp. 130–131) describe this hollow cylinder as two layers ot cells thick and composed of groups of spore-mother-cells separated by clusters of sterile cells that later become the elaters. The writer's preparations indicate far less regularity of structure than this description would imply. In many cases the spore-mother-cells formed a single layer around the columella and the amount of sterile tissue was very insignificant. Only occasionally was the layer of cells distinctly double and then very irregularly so. There appears, therefore, to be considerable variation in the complexity of the sporogenous tissue in Anthoceros, and the simplest conditions approach very closely the arrangement of cells reported by Campbell (**'98**) for Dendroceros.

The cubical cells of the archesporium frequently contain nuclear figures, but the spindles are very small and unsatisfactory for study. Such a spindle is shown in *fig. 1*, with the chromosomes separating into two groups and about to pass to the poles. The cytoplasm is granular and contains minute vacuoles. The writer was not able to find a trace of the chloroplast in these cells, but looks forward to a further study of this point in living material and with special methods of staining.

The spore-mother-cells become very quickly differentiated by a rapid increase in size. Their nuclei are not at first to be distinguished from those in the cells of the archesporium, for they are small and contain the one prominent nucleolus and an ill-defined network of linin threads. The most conspicuous change in the cell is the appearance in fixed material of the chloroplast. This structure is at first a slightly denser mass of protoplasm than the other contents of the cell, but so small that it can scarcely be distinguished from differentiated granular regio of the cytoplasm. Indeed, the first clear proof of its presen in my preparations has been the sharp staining of the starc grains (purple with gentian violet) that are found almost imm diately in the interior. The reader may see in *fig. 3* such early stage in the development of the chloroplast, and sho notice that the three small starch grains lie in a homogeno matrix of much the same consistency as the surrounding cy plasm. The chloroplast in cells younger than that shown *fig. 3* could not be recognized with certainty by the methods staining employed in the present study.

The chloroplast once differentiated soon becomes the m conspicuous object in the spore-mother-cell by virtue of its s and brilliant differentiation with stains (*figs. 4, 5*). The pec arities become more apparent as it enlarges. The outline sharply marked but it has been impossible to establish a dist bounding membrane. The interior contains beautifully def starch grains that become very numerous as the chloropla increases in size and finally fill by far the greater part of enclosed region. Each starch grain at first occupies a lit cavity surrounded by delicate films of protoplasm that app as strands in the sections figured (*figs. 5–9*). The chloropla has therefore a honeycomb-like structure, each cavity be entirely or almost entirely filled by a grain of starch.

The structure of the protoplasmic strands and films aro the starch grains proved a very difficult subject for study. Th are granular and extend on all sides to the boundary of chloroplast where they merge into the densely staining film the exterior. But the bounding film grades off insensibly the cytoplasm and it was not possible to distinguish by any sta reaction the strands inside the chloroplast from the protopla outside.

The first division of the chloroplast takes place very quick after the structure reaches a certain size. Preparation for process is usually indicated by the chloroplast elongating a bending around the nucleus in the form of a thick cresce

The phenomenon of division is one of simple constriction in the middle region and final separation of the halves as the furrow deepens around the chloroplast. It is very plain that the line of fission is determined by the furrow and not from the interior of the chloroplast. Therefore if any particular region of the protoplasm is actively concerned in this process one would naturally first seek the agent in the peculiar film bounding the chloroplast. However there is little in the structure of this region to indicate such important activities.

After the first division of the chloroplast the two portions usually separate and pass to opposite ends of the elongated spore-mother-cell (*fig. 6*). Each has a structure quite similar to the parent chloroplast except that the starch grains become even more numerous and prominent and the fine strands and films of protoplasm separating the starch grains are less clearly defined. Soon after this first division of the chloroplast there takes place that peculiar condition of the nucleus termed synapsis, but this phenomenon had best be considered in a later portion of the paper that deals with nuclear activities. There is a well defined period when the spore-mother-cell has only two chloroplasts, and no longitudinal section of the sporogonium will fail to exhibit a region in which all of the cells are in this condition. As a spore-mother-cell grows older, and increases still further in size, indications of the approaching second division of the chloroplast become manifest.

The second division of the chloroplast repeats exactly the phenomenon of the first division. As a rule both structures concerned are active at the same time, but one may be in a more advanced state of fission than the other. The proportions of the spore-mother-cell at this time are more nearly equal (*fig. 7*) than in the previous condition, *i. e.*, the cell is broader in proportion to its length. As a result the four chloroplasts are distributed around the centrally placed nucleus in such a manner that each occupies the greater part of one of the four tetrahedral divisions of the spore-mother-cell that are present after the separation of the cell contents to form the spores. Sometimes the second division of the chloroplast follows so closely after th first that the final products are arranged for a short time in t form of a bow around the nucleus (*fig. 9*). Such conditio are however only transitory and result from the elongated form of such cells.

The structure of the four daughter chloroplasts exhibits th difference from that of the mother structure shown in *figs. 4* The protoplasmic material that formerly filled the spaces betwee the starch grains as a spongy network of strands and films no appears to be entirely absent or present only in a much reduce form. The chloroplast has the structure of a vesicle filled wit starch grains. It is interesting to contrast this condition with th conditions exhibited in the youngest spore-mother-cells. Ther the bulk of the chloroplast was made of protoplasm and the fe starch grains were insignificant. But in the oldest spore-mothe cells the chloroplast has become one great storage vesicle starch, conspicuous by the absence of that protoplasmic differe tiation which is so characteristic of the chloroplast whereve found.

The problem of the division of the chloroplast and the dis tribution of the resultant daughter plastids through the cell full of interest. Does the development of the furrow and th final fission of the chloroplast result from the activity of th protoplasm in or around the structure? It is conceivable tha the division has its cause merely in the advantage that migh come from a more even distribution of the chlorophyll throug the cytoplasm. Thus it is obvious that the arrangement show in *fig. 5* is very unsymmetrical. Perhaps a strain may be exerte upon a chloroplast bent around the nucleus in such a manne and finally result in its fission, and then lead to a redistributio of the cell contents to bring about a certain balance in the ce The difficulty of this view as applied to Anthoceros lies in th fact that the number of chloroplasts is fixed at four, correspond ing exactly to the number of spores. How could a numerica coincidence so important in phylogeny be left to the play o forces merely acting for symmetry and the advantageou distribution of chlorophyll throughout the cell? But if we assign the cause of the division to the protoplasm in or around the chloroplast there arise complications very difficult to explain. The four chloroplasts are in the cell long before the nucleus divides. Can it be supposed that cytoplasm would be intrusted with so important a task as the preparation of a chloroplast for each of the four nuclei that are later to preside over the spores before there is any indication that such nuclear division is to take place? The process reverses what would appear to be the natural course of events; *i. e.*, one would suppose that the division of the nucleus would determine the position of the four spores and that the cell contents would arrange themselves later with reference to the nuclei. Perhaps this really is the fact, and it may be merely chance that each of the four nuclei finds a single chloroplast to accompany it in the spore, but such coincidence of numbers would be very curious. A somewhat similar problem is presented by the oospore of Coleochaete, where, according to Oltmanns, eight chloroplasts are formed before the nucleus divides, and then as cell division progresses the nuclei are distributed symmetrically until each becomes associated with one chloroplast.

The structure and behavior of the chloroplast suggests some extremely interesting lines of research. Is there such a substance as plastidplasm, a particular form of protoplasm with morphological characters that may distinguish it from trophoplasm, filarplasm, centrospheres, and other differentiated structures in the cell? If there is a plastidplasm, what form does it assume at the periods of ontogeny, when chlorophyll and other pigments are absent? Is the plastid a permanent organ of the cell, as is perhaps generally supposed? As the plastid is a region of the protoplasm where the pigment is gathered, and consequently the seat of metabolic activity, it is possible that these functions might produce its form and structure. It is even conceivable that the differentiation of a plastid may not lie in peculiarities of protoplasm itself, but represent the outward effects of the metabolic phenomena concerned with its pigment.

It appears to the writer that plant cytology has a very important field open for investigation dealing with the structure of the chromatophore and its place in ontogeny.

We pass now to the second part of the paper, which is to consider the behavior of the nucleus in sporogenesis. The structure of the spore-mother-cell and process of nuclear division in Anthoceros was first described by Strasburger (**'80**, p. 162). The following account entirely supports the essentials of those observations, but the present investigation attempts a more detailed examination of nuclear activities, involving the problems of spindle formation, synapsis, the succession of nuclear divisions, and formation of the walls between the spores.

The fact has already been stated that eight chromosomes are present in the sporophyte, and consequently enter the nucleus of the spore-mother-cell. This nucleus in a resting condition is very similar to the nuclei of the archesporium and shows very little structural differentiation (*fig. 3*). It is small and its linin network is very inconspicuous, but the nucleolus is prominent.

Coincident with the appearance and increase in size of the chloroplast the nucleus enlarges (*figs. 4, 5, 6*) and the threads of linin become very prominent. They are so exceedingly small, however, that it was not possible to determine accurately the structure, even with a Zeiss apochromatic immersion lens under the magnification of 2250 diameters. Dark-staining minute bodies along the linin thread are presumably granules of chromatin, but their distribution could not be ascertained with certainty, nor was it possible to follow the convolutions of the spirem.

The condition of the nucleus after the first division of the chloroplast is shown in *fig. 6*. The spirem thread is strongly developed, but the spotted character of the nucleolus indicates that the latter structure may be the seat of internal changes. Suddenly, as indicated by the rarity of intermediate conditions, the nucleus passes into the state of synapsis. The spirem thread contracts into an irregularly shaped mass that usually lies by the side of the nucleolus. This condition is illustrated in *fig. 7*. Synapsis in Anthoceros seems to occur after the first division of the chloroplast, and it is very apt to persist through to the second division. The contracted threads of linin then gradually extend outward through the nucleus (*fig. 8*), and finally a spirem condition is attained similar in all outward appearances to that present in the nucleus before synapsis.

The nucleolus after synapsis is often found somewhat fragmented, as is shown in *fig. 10*. Whether or not the nucleolus partly dissolves and contributes material to the linin thread, as has been suggested for certain forms (*e. g.*, Lilium), could not be determined for Anthoceros.

Synapsis in Anthoceros does not appear to the writer to be an artifact, and in this opinion he agrees with the views expressed by a number of investigators who have studied this phenomenon in other types. Particularly favorable opportunities are presented for the solution of this problem in Anthoceros, because one may subject at one time all conditions of the spore-mother-cells to the same fixing fluid. Under these conditions synapsis always appears in that particular region of the sporogonium comprising cells in the condition just before and during the second division of the chloroplast. No nuclei were ever observed in synapsis in other parts of the sporogonium, and nuclei in younger and older cells immediately adjoining the disturbed region presented the typical fully expanded spirem thread. Yet all of these cells had been bathed in the same fixing fluid and experienced identical treatment in the preparation of the slides. Of course it may be claimed that the contents of the nuclei are more subject to shrinkage at the time when synapsis appears, but it should be understood that such a variety of fixing fluids as Flemming's, picro-acetic, corrosive sublimate, chrom-acetic, and Merkel's, all gave identical results. Unfortunately the small size of the nuclear elements in Anthoceros made a detailed study of this interesting process extremely difficult, and led to no definite conclusions as to the meaning of synapsis.

We have seen that events take place during the growth of the spore-mother-cell in the following order: *first*, an increase in size of the nucleus, the assumption of the spirem condition, and the first division of the chloroplast; *second*, sudden synapsis at the time when there are two chloroplasts in the cell, and often continuing until after the second division of these structures; *third*, gradual emergence of the nucleus from synapsis. These changes present the spore-mother-cell ready for nuclear division with the following arrangement of the cell contents. The four chloroplasts are distributed symmetrically, with the nucleus in the center of the cell. In this condition the cell remains for a period somewhat longer than that of the synapsis, and then prepares for the first division of the nucleus.

It is difficult to recognize the earliest indications that the nucleus is approaching prophase of division. A somewhat later stage is very conspicuous, when the outline of the nucleus becomes angular and a mat of delicate threads surrounds the structure (*fig. 12*). There is a period, however, previous to this condition, when the nuclear membrane appears much less firm and somewhat irregular in outline. This structure follows the spirem stage such as is shown in *fig. 10*, and precedes the unmistakable prophase conditions illustrated by *figs. 12, 13*. Its appearance is given in *fig. 11*, and the following peculiarities should be noted, viz., an irregularly outlined nuclear membrane, a faint linin network, and fragmentary nucleolus. But perhaps the most important characteristics appear outside of the nuclear membrane in the cytoplasm as a delicate web of fibrils closely applied to the nucleus. These fibers are so delicate as almost to defy the reproduction that has been attempted in *fig. 11*. They presage the development of the spindle.

In the prophases the nucleus may exhibit some extremely varied appearances. The form is very irregular, taking on curious wavy angles. Outside of the nucleus there is a region of protoplasm manifestly very different in structure from the neighboring cytoplasm. In overstained preparations this region is very conspicuous, forming a sort of zone around the nucleus, but the details of its structure can only be made out in very favorable preparations. Then it is seen to possess a fibrillar structure. The delicate threads lie all around the nucleus, but they accumulate conspicuously at the pointed projections (*figs. 12, 13*).

At this time one is apt to find the linin network contracted away from the nuclear membrane and gathered in the form of a confused tangle in the central region somewhere near the nucleolus (*fig. 13*). The chromatic material becomes more prominent and from the ill-defined mass in the interior there emerge four deeply staining chromosomes (*figs. 12, 14*). When the chromosomes are fully differentiated one may expect to find one or two of the angles of the nucleus more prominent than the others, and the fibrils around them taking on the appearance of spindle fibers (*figs. 13, 14*). The process of spindle differentiation is a gradual one, and only in the later stages is it possible to feel sure of the direction that the axis will assume. It is seldom that the two poles appear from the beginning so nearly opposite one another as to have a common axis. It is more usual for the spindle to be somewhat bent at first, as is shown in *fig. 14*. However, ultimately the two poles arrange themselves to form a symmetrical spindle of the form illustrated by *figs. 15, 16, 17, 18*.

The fully developed spindle has an interesting structure, with several clearly marked features well shown by *fig. 18*. The poles are flat, or perhaps slightly convex; they are never pointed. Mantle fibers are conspicuous around the nuclear plate (*figs. 16–18*), but what relation these bear to the chromosomes could not be determined.

In view of the condition reported by Farmer ('94 and '95) for a number of the Hepaticae, notably Pellia, Pallavicinia, and Fossombronia, it became a very important problem to determine whether or not centrospheres were ever present at the poles of the spindles. The writer searched his preparations with great care for these structures, but came to the conclusion that they do not exist in Anthoceros. Sometimes the cytoplasm around the spindle contains large deeply staining granules, such as are shown in *fig. 17*, and these may occupy positions near the ends of the spindles, but extensive observations would convince anyone that they have nothing whatever to do with centrosomes. The protoplasm in the vicinity of the pole is often very dense in structure, containing no granules and few vacuoles. It frequently presents the appearance shown in *fig. 16*, but it never exhibited indications of that differentiation expected of a centrosphere.

It will be apparent to the reader from this account that the spindle of Anthoceros during the first division follows a history closely parallel to that described for homologous cells in Lilium by Mottier ('97), Hemerocallis by Juel ('97), Equisetum by Osterhout ('97), and Cobæa by Lawson ('98). That is to say, the spindle is organized by numerous delicate fibrils of protoplasm that develop conspicuously during prophase in the cytoplasm around the nuclear membrane. The fibrils are at first somewhat irregularly distributed, but finally become arranged in the form characteristic of the respective spindles.

Strasburger in the third edition of the *Lehrbuch der Botanik*, 1898, p. 67, has introduced the term filarplasm to be applied to protoplasm having the form and activities above described and so clearly established by the researches of his students during the past three years. The writer understands that filarplasm is supposed to be made up of the substance designated by the older term kinoplasm. However, filarplasm has morphological characters, as indicated by its name, and these are the thread or fibril-like structures. The term therefore expresses admirably the facts of morphology without implying or assigning physiological activities to the substance.

It will be very gratifying if future investigation should establish filarplasm as an element in the cells of higher plants differentiated from other forms of protoplasm. This investigation appears to extend the range of mitoses associated with filarplasm into a group of plants much lower than the lowest previously reported (pteridophytes by Osterhout). It is also significant that it should be a class containing one large order, the Jungermanniaceæ, where according to Farmer centrospheres are very marked features of the mitotic figures. There should be types of plants that will allow us to form some idea of the relationship between filarplasm and the asters and centrospheres present in certain thallophytes, in other words solve the problems of their places in phylogeny. Perhaps the future study of Hepaticæ will throw some light on this question.

The two sets of daughter chromosomes that result from the division at metaphase pass very quickly from the nuclear plate to the poles of the spindle. Here they may frequently be found clustered together in a region of dense protoplasm that surrounds the group, as is shown in *fig. 19*. The development of a nuclear membrane around the chromosomes finally organizes the two daughter nuclei which are connected for a short time by the spindle fibers. These latter structures gradually fade away, beginning at the poles (*fig. 20*), and finally entirely disappear. The daughter nuclei then lie in undifferentiated cytoplasm usually occupying positions between the chloroplasts somewhat as is indicated in *fig. 21*.

The daughter nuclei following the first division pass into a fully developed resting condition. The chromosomes break up into a number of chromatin granules that become distributed over a linin network. A prominent nucleolus appears. In sections of a sporogonium one may always find spore-mother-cells containing two nuclei, although this condition is quickly replaced by the succeeding second division.

The second mitosis in the spore-mother-cell involves both nuclei simultaneously, but the two nuclear figures are developed independently of one another. The two spindles are never united by cross fibers. Sometimes the two spindles will lie almost side by side, but usually they are placed at right angles to each other. Frequently one section of a spore-mother-cell will give a polar view of one spindle and the neighboring section a longitudinal view of the other. An instance of this character has been chosen to illustrate the principal features of their structure and is shown in *fig. 24*. By comparing *fig. 24* with illustrations of the first division (*figs. 16–18*) it will be seen that the spindles of the second mitosis are much smaller. However they exhibit an essentially similar structure, having flattened poles without centrospheres. Several prominent fibrils make up the center of the spindle which is bordered by an ill-defined set of mantle fibers. The polar view of a spindle shown at the left of *fig. 24* demonstrates that four chromosomes are present at the nuclear plate the same number that appears during the first mitosis. Prophase and anaphase conditions of the second mitosis are found only with great difficulty and are very unsatisfactory for study because of the small size of the elements involved.

The four nuclei that result from the second mitosis associate themselves each with one chloroplast, and with these become distributed symmetrically through the cell, so that the protoplasm naturally segregates into four regions representing what are later to become the tetrahedral division of the spore-mother-cell (*fig. 26, 27*). The spindle fibers disappear completely.

The problem of the splitting of the chromosomes engaged the writer's attention, but it must be plain that Anthoceros is not a favorable subject for the study of this process. The nearly spherical form of the chromosomes offers immense difficulties in orientation. Farmer (**'95**) reported some peculiar conditions in the forms studied by him, which he considers as illustrations of the "heterotype" division described by Flemming. They result from the habit that the chromosomes have of doubling on themselves and then being pulled apart as **V**-shaped daughter chromosomes. The figures are very complex, but Farmer assures us that the division is really longitudinal and not transverse, so that it cannot be interpreted as qualitative. In Anthoceros all evidence that the writer can present indicates that the splitting in both mitoses is longitudinal and after the normal type. Polar views of spindles after the splitting of the nuclear plate sometimes present the daughter chromosomes, eight in number, arranged in four pairs. Such a stage is shown in *fig. 23*, and the grouping certainly indicates that each of the original four chromosomes has divided longitudinally into halves. It is not at all unusual to find the two sets of daughter chromosomes placed as in *fig. 22*, which might suggest transverse fission, but the writer is not willing to accept this evidence, as such an arrangement would naturally appear when the daughter chromosomes pass away from the nuclear plate to the poles of the spindle.

We have now finished our account of nuclear activities in the spore-mother-cell, but there remains for consideration the description of the manner in which the cell contents are divided to form the spores. After the two mitoses each of the four nuclei lies in a region of dense protoplasm at the side of a chloroplast towards the interior of the cell (*fig. 25*). The bulk of the protoplasm is therefore collected into four masses somewhat apart from one another but connected by very numerous delicate filaments. The cross filaments are very conspicuous but irregular in their arrangement, frequently anastomosing. As shown in *fig. 25* they are not confined to the vicinity of the nuclei but connect all portions of the separated regions of protoplasm. They do not resemble spindle fibers, being much thicker, but have instead the appearance of strands of cytoplasm. Following the condition shown in *fig. 25* one may find stages similar to *fig. 26*. It is plain that the protoplasmic strands have spread sideways and fused with one another so that there is now present a film of protoplasm between tetrahedral regions of the spore-mother-cell. This film marks exactly the position that is finally to be occupied by cell walls when the spores are fully organized.

The peculiarity of the process just described lies chiefly in what seems to be its entire independence of spindle fibers. It is the generally accepted view that the walls crossing spore-mother-cells and pollen-mother-cells are derived from cell plates that result from the fusion of spindle fibers after anaphase. The writer thinks that no investigator has described a condition similar to Anthoceros. The present studies indicate that the spindles of the two successive mitoses completely disappear. This is certainly a very difficult point to determine, but the writer feels confident that the anastomosing strands which connect the four masses of protoplasm in the spore-mother-cell, as has been shown in *figs. 25, 26*, are formed entirely independently of spindle fibers. How then are the walls formed?

It is certain that the film of protoplasm indicated in *fig.* 2 thickens and finally gives place to a straight wall, at first delicate (*fig. 27*) but gradually becoming firmer until its cellulose nature is unmistakable.

The phenomenon is typical of one of the processes recently discussed by Strasburger ('98), in which a cell wall is formed in the interior of the protoplasm. It appears as if the film of protoplasm exhibits the activities present in the "Hautschicht" when it lays down or increases the thickness of a cell wall in the manner known as apposition, involving, at least in part, the change of its own substance into cellulose.

With the separation of its contents the spore-mother-cell as a unit ceases to exist, and a new set of activities begins that may very properly be reserved for discussion in another paper. Some interesting events take place in the spore as it ripens, but technical difficulties interfere greatly with their elucidation.

The peculiar fact that the sporogonium of Anthoceros presents spore-mother-cells in all stages of development makes it possible to contrast the times occupied by the various changes. One cannot establish the actual duration of any process, but within certain limits it is possible to determine the relative periods of each event. It must be assumed that the rate of growth during the season is approximately uniform. The unit of the calculation must be the time necessary to differentiate one spore-mother-cell from the archesporium. As the result of a laborious examination and count the writer ventures to present the following time schedule of the events which take place in the spore-mother-cell. From 12-18 units of time are necessary to bring the spore-mother-cell to the period of the first division of the chloroplast. The division of the chloroplast occupies 6–10 units. The nucleus is in synapsis 12–20 units. For 30–50 units the cell has two chloroplasts. It takes about 25 units after synapsis to produce conditions favorable for mitosis. The first mitosis occupies 1–3 units, and the two daughter nuclei rest for 2–4 units before the second mitosis which is more rapid than the first. There is finally a period of 3–6 units before the spores are definitely organized by the partition walls across the spore-mother-cell.

The results presented by this investigation indicate that the Hepaticæ are likely to furnish some very interesting material for future researches in cytology. From superficial examination it appears probable that the Ricciaceæ and Marchantiaceæ in general present mitoses similar to Anthoceros, but we may hope that some forms exist that will harmonize the peculiarities described for the Jungermanniaceæ with the conditions found in Anthoceros.

EXPERIMENTAL TECHNIQUE.

As stated in the beginning of the paper the writer experimented with a number of fixing agents to determine as precisely as possible their merits or faults. Of these Flemming's formula designated "weak" gave decidedly better results than any other. In Anthoceros, and presumably for other types, the chief test of a fixing fluid is its effect upon the achromatic parts of a nuclear figure. Chromosomes are the least difficult of all the nuclear elements to preserve. Preparations will not infrequently present beautiful views of nuclear plates when the spindles are manifestly in very bad condition. The following is a brief statement of the effects of several fluids upon nuclear figures in the spore-mother-cell.

Chrom-acetic acid fixes filarplasm but the safranin stains diffusely after it, and gentian violet does not hold well in the spindle fibers. If sections fastened to the slide be left several days in weak Flemming the staining qualities with safranin and gentian violet are much improved although it is doubtful if they can be made as good as those presented by Flemming fixed material.

Merkel's fluid (1 per cent. chromic acid 12^{cc}, 1 per cent. platinum chlorid 12^{cc}, water 72^{cc}) even when used for long periods (36 hours) is thoroughly unsatisfactory. The spindles are badly fixed.

Boveri's picro-acetic acid gives beautifully bleached tissue but achromatic regions are not clearly differentiated although chromatic elements stain well.

Sublimate-acetic (5 per cent. glacial acetic acid in saturated solution of corrosive sublimate) is not good. Nuclear membranes and filarplasm are very poorly preserved.

Hermann's fluid is very much like Flemming's in its effects and is thoroughly satisfactory.

The osmic acid of the Flemming's and Hermann's mixtures appears to give them certain advantages over all other fluids. Although they may not kill and preserve tissue better than some other agents, as for example chrom-acetic acid, certain stains, safranin and gentian violet, differentiate all structures of the cell very much better when they have been used.

SUMMARY.

The number of chromsomes is eight for the sporophyte and four in the gametophyte.

The chloroplast appears rather suddenly in the spore-mother-cell as a differentiated region of the protoplasm, containing several starch grains. When fully developed it has a honeycomb structure, each cavity being occupied by a grain of starch.

The division of the chloroplast is one of simple fission, apparently through forces acting outside of the structure and perhaps concerned with the film of protoplasm that surrounds it.

Synapsis occurs in the nucleus soon after the first division of the chloroplast. It is not an artifact.

The second division of the chloroplast presents the spore-mother-cell ready for the division of the nucleus. The four chloroplasts, hardly more than vesicles filled with starch grains, are arranged symmetrically in the cytoplasm with the nucleus in the center of the cell.

The resting nucleus has a nucleolus and a spirem thread, which however, is so small that details of structure could not be determined.

Prophase conditions of the nucleus show the presence of a mesh of delicate fibrils (filarplasm) around the structure. The nuclear membrane is at first irregularly angular, but finally two poles of a spindle are differentiated.

The metaphase presents a spindle with flattened poles, entirely lacking bodies that might be interpreted as centrospheres or centrosomes.

There is a period of rest after the first mitosis when each daughter nucleus has a nucleolus and spirem thread. The two mitoses are, therefore, successive.

The second mitosis presents structural features quite identical with the first.

The chromosomes are four in number in each mitosis. They appear to split longitudinally.

All traces of the spindles become lost soon after each mitosis.

The fully mature spore-mother-cell presents four chloroplasts, each with a single nucleus on the interior side. The protoplasm segregates in these four regions of the cell, leaving spaces that are crossed by many anastomosing strands of cytoplasm. These strands cannot be traced from spindle fibers and appear to have no connection with filarplasm.

The walls separating the cell contents into four spores are derived from films of protoplasm that appear between the chloroplasts with their respective nuclei. The films are formed by the coalescence of strands of cytoplasm that cross the spaces between the four regions of cell contents.

The University of Chicago.

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EXPLANATION OF PLATES IX AND X.

All preparations were studied under a Zeiss oil immersion 2^{mm} aper. 1.30 with compensation-oculars. Figures were sketched with an Abbé camera at the following magnifications, *figs. 3–7, 9, 10, 12, 16–21, 24–27*, magnified 1000 diameters; *1, 2, 8, 11, 13, 14, 15, 22*, 1500 diameters and *fig. 23*, 2250 diameters.

All preparations except that shown in *fig. 2* from material fixed in weak Flemming, sectioned 5μ thick and stained on the slide; *figs. 1, 15, 18, 22* and *23* from slides stained with iron-alum-haematoxylin after the method of Haidenhain; all other figures from preparations stained with Flemming's triple stain, safranin, gentian violet, and orange *G*.

PLATE IX.

Fig. 1. Nuclear figures from archesporium, just after the splitting of the chromosomes whose number is large, apparently about sixteen, eight for each daughter nucleus.

Fig. 2. Nuclear figure from antheridium, four chromosomes.

Fig. 3. Spore-mother-cell, early condition of chloroplast with three or four grains of starch.

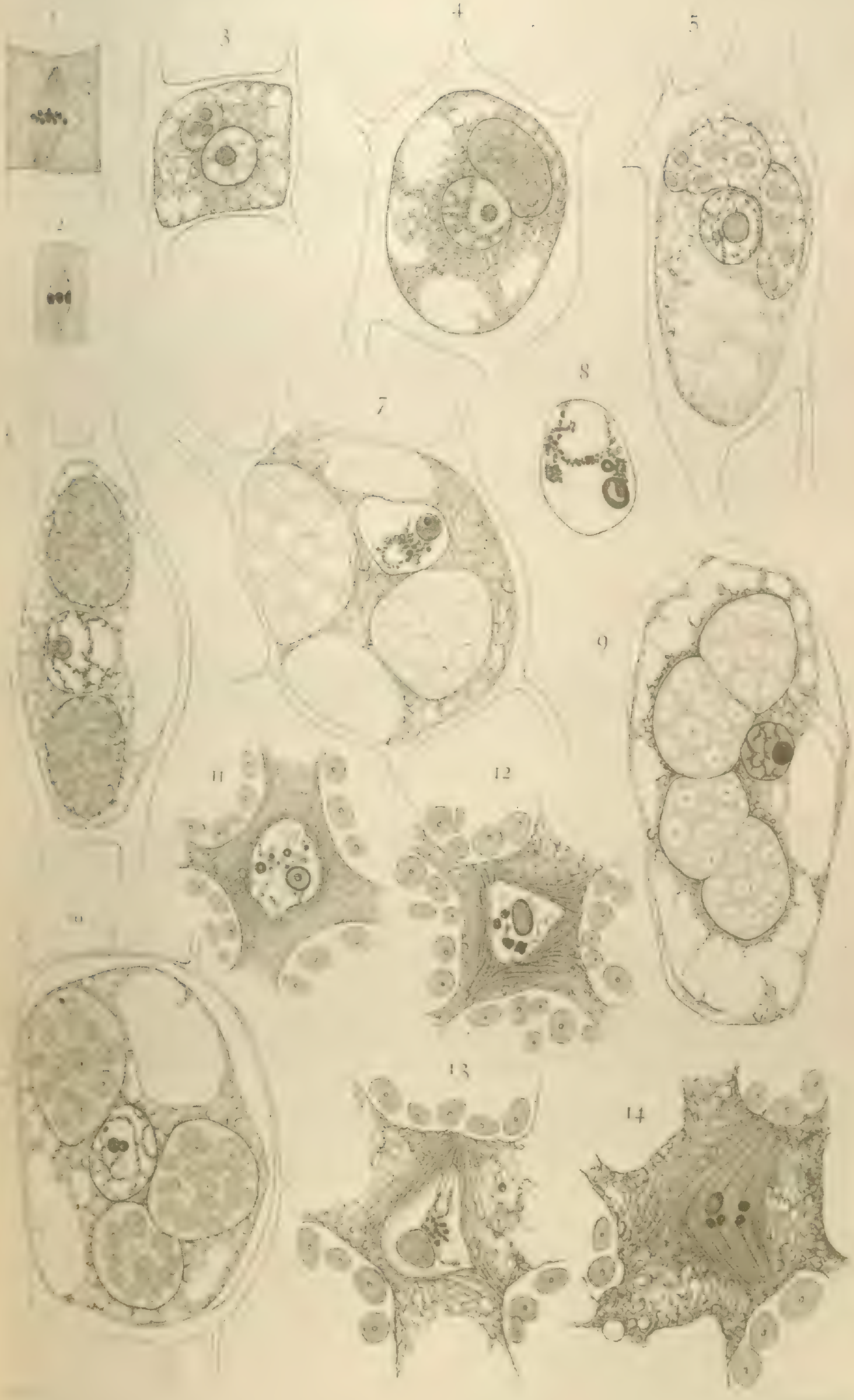
Fig. 4. Spore-mother-cell in more advanced condition than *fig. 3*, linin network more prominent, chloroplast elongating preparatory to division; starch grains prominent.

Fig. 5. First division of chloroplast.

Fig. 6. Two chloroplasts, linin network prominent.

Fig. 7. Synapsis, two chloroplasts.

Fig. 8. Nucleus emerging from synapsis.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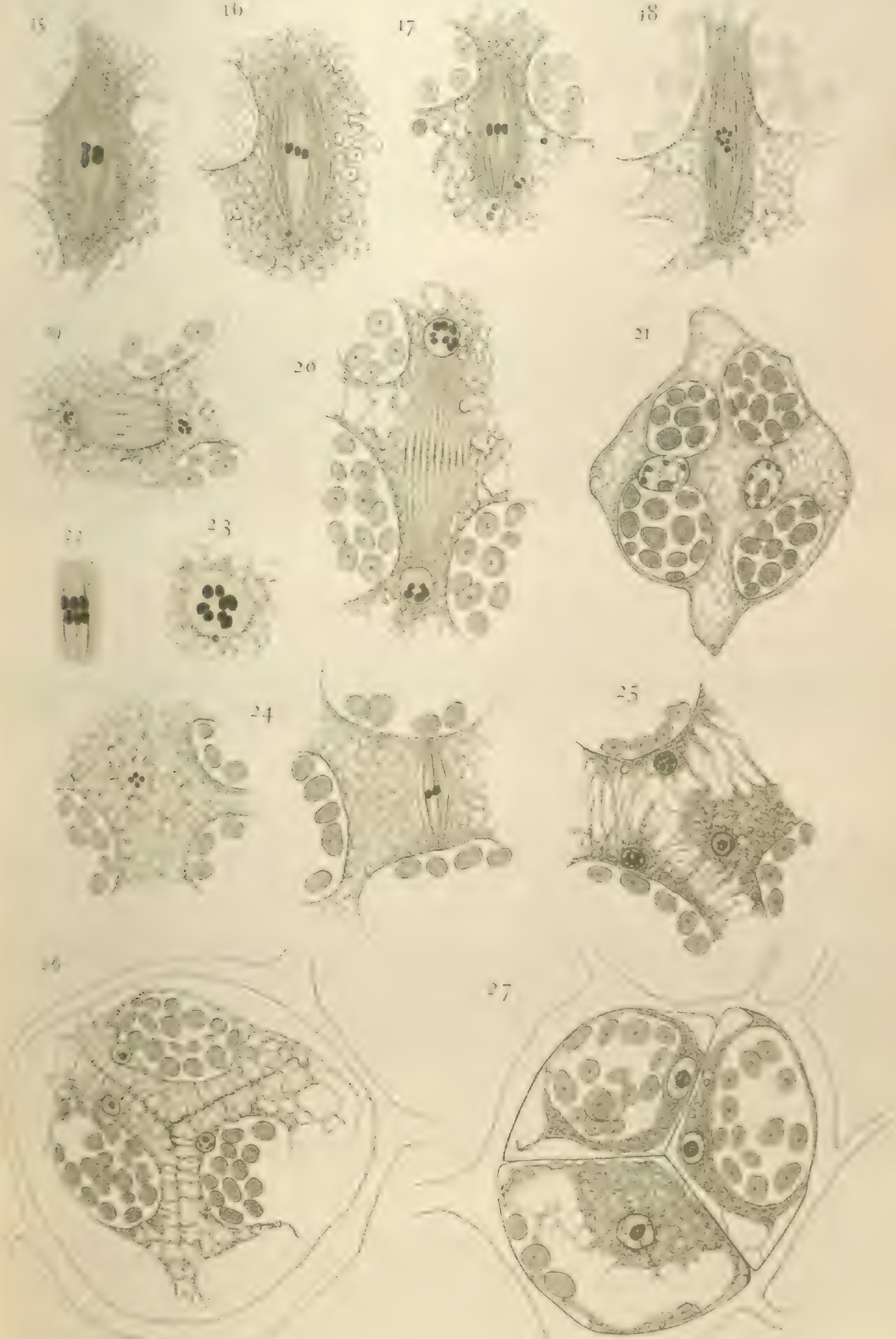
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DAVIS on ANTHOCEROS.

FIG. 9. Second division of the chloroplasts.

FIG. 10. Spore-mother-cell at maturity, four chloroplasts.

FIG. 11. First indication of approaching mitosis, accumulations of filarplasm around the nucleus whose membrane appears less clearly defined.

FIG. 12. Prophase, filarplasm well differentiated.

FIG. 13. Prophase, one pole of spindle developed.

FIG. 14. Late prophase, the two poles of the spindle differentiated, four chromosomes assembled in the equatorial region.

PLATE X.

FIG. 15. Early metaphase, four chromosomes at nuclear plate.

FIG. 16. Metaphase, strongly developed mantle fibers.

FIG. 17. Metaphase, granular cytoplasm around the nuclear figure.

FIG. 18. Late metaphase, after splitting of the chromosomes, very clearly defined spindle.

FIG. 19. Anaphase, spindle still clearly defined.

FIG. 20. Telophase, trace of spindle in equatorial region.

FIG. 21. Spore-mother-cell, daughter nuclei after first mitosis in resting condition, four chloroplasts.

FIG. 22. Nuclear plate at first mitosis, side view.

FIG. 23. Nuclear plate of second mitosis viewed from pole of spindle, indicating longitudinal splitting of the chromosomes.

FIG. 24. Adjacent sections of same spore-mother-cell during second mitosis; nuclear plate of one spindle with four chromosomes viewed from the end; side view of the other spindle.

FIG. 25. Center of spore-mother-cell showing three nuclei, cytoplasm segregated around the nuclei and chloroplasts, delicate strands connecting the regions.

FIG. 26. First indication of the position of the walls that are to be formed in the spore-mother-cell; the coalescence of connecting strands of cytoplasm to form a delicate film.

FIG. 27. Final separation of the protoplasmic contents of the spore-mother-cell.

THE STRUCTURE AND DEVELOPMENT OF CRYPT MITRIUM TENERUM.

LE ROY ABRAMS.

(WITH SIX FIGURES)

THE genus Cryptomitrium, represented by the single spec *C. tenerum* (Hook.) Austin, has not yet been thoroughly stud and as the plant has been collected in several localities ne Stanford University, at the suggestion of Dr. Campbell a st of its structure and development was undertaken, in order possible, to determine its relationship to the other Marchantiace

The first collections were made late in the spring of 18 at which time the plants were mature, and the spores alm ripe. Some of these plants were placed in alcohol, wh others were allowed to become dry, and the earth upon wh they were growing was kept until the following Septemb when the work upon the plant was taken up. A considera number of the mature sporogonial receptacles were also put dry, in order to study the germination of the spores.

Some of the pieces of earth with the dried specimens up them were thoroughly soaked, and then kept well moisten Within a day or two the tips of the apparently dried-up pla became green and fresh, and in about two weeks the anther began to form. All of the material for study was obtain in this way, until after the rains came. Then considera material was collected from out of doors, where it develop much faster and was healthier than that grown in the lab atory.

THE THALLUS.

Cryptomitrium, like most other Marchantiaceæ, has a fl dichotomously branched thallus, which in this species is ve thin and delicate. The smooth glossy appearance, by wh one can easily distinguish the sterile plants from *Fimbri*

[s

Californica Hampe, a species almost always associated with it, is due for the most part to the minute stomata.

These stomata are surrounded by eight, occasionally seven, very symmetrically arranged guard cells (*a, fig. 1*), and not by five or six, as stated by Stephani.[1] Each opens into a well-developed air chamber (*b, fig. 1*), the boundaries or walls of which can be seen easily with a hand lens, forming a fine network under the epidermis.

These air chambers are much the same as in *Fimbriaria Californica*. They are distributed irregularly throughout the green tissue. Only a single layer of cells separates them, and often one cavity is connected with another. Their development begins a little further back from the apical cell than in the above mentioned species as described by Campbell.[2]

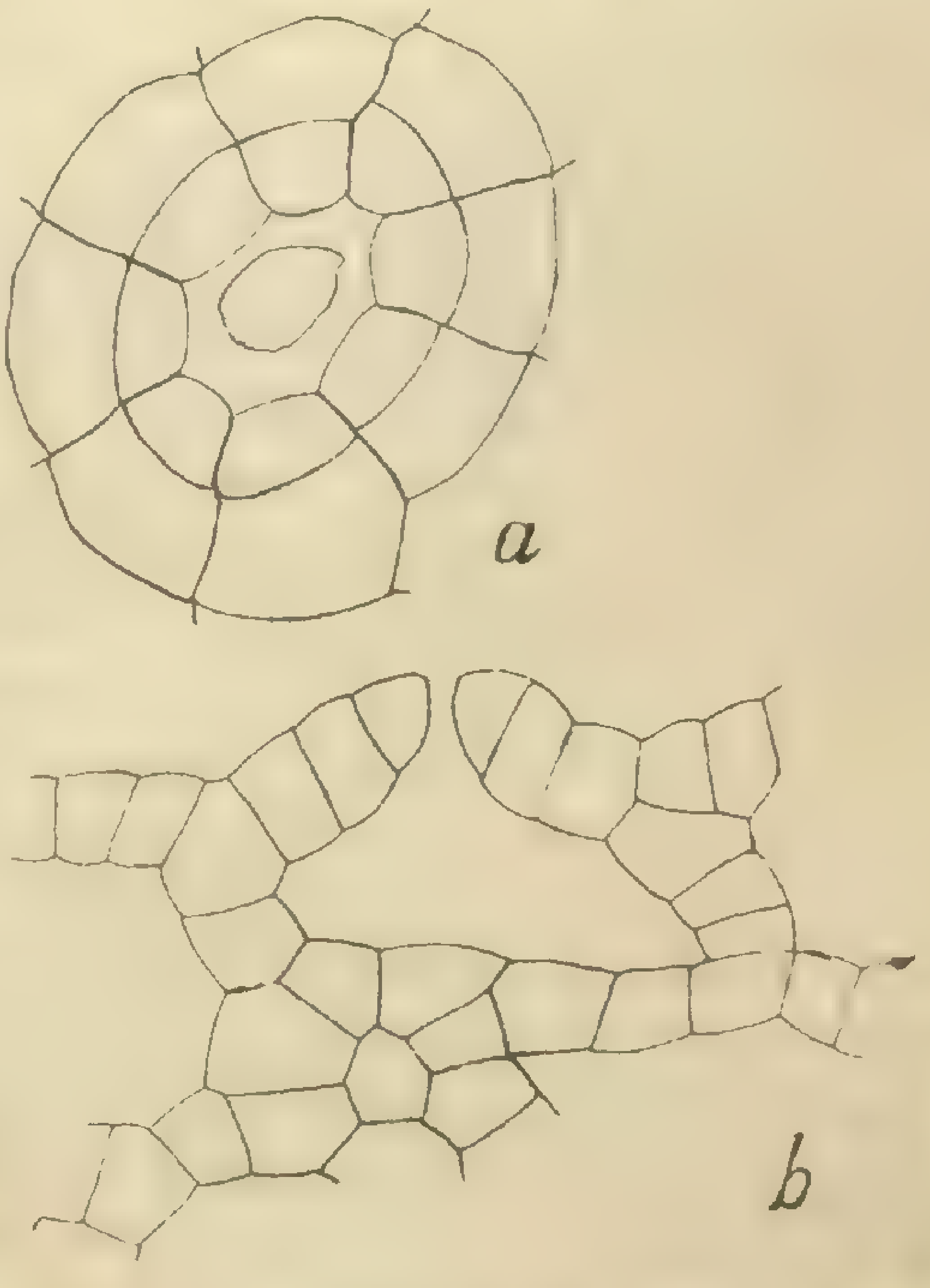

FIG. 1.—Stomata of the thallus. *a*, as seen from the surface. ×600. *b*, transverse section. × 600.

The general appearance and external characters of the ventral scales have been quite thoroughly and accurately described by Stephani (*loc. cit.*), and as their development does not differ from that of other allied genera it need not be repeated here.

As might be expected, both kinds of root hairs, those with smooth thin walls and those with tuberculate walls, which are characteristic of the Marchantiaceæ, are present.

The peculiar oil bodies found in so many of the Hepaticæ were found scattered throughout the thallus, ventral scales, and sporogonial receptacle. The development and composition of these oil bodies found in the Hepaticæ have been thoroughly studied by Pfeffer.[3]

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[1] Bot. Gaz. 17: 58–60. 1892.

[2] Campbell, D. H.: Mosses and Ferns, p. 48. 1895.

[3] Pfeffer, W.: Die Oelkörper der Lebermoose. Flora 32: ——. 1874.

THE SEXUAL ORGANS.

Cryptomitrium tenerum is monœcious. The antheridia form single row just back of the sporogonial receptacle. Th are sunk deep in the thallus, and each one is marked on t surface by a small conical ostiolum. These ostiola are ve inconspicuous, however, and their presence can scarcely detected with a hand lens.

The antheridia are developed before the female receptac and in Fimbriaria and other allied genera, they are formed on th dorsal side just back of the apical cell (*a*, *fig*. 2). The primar

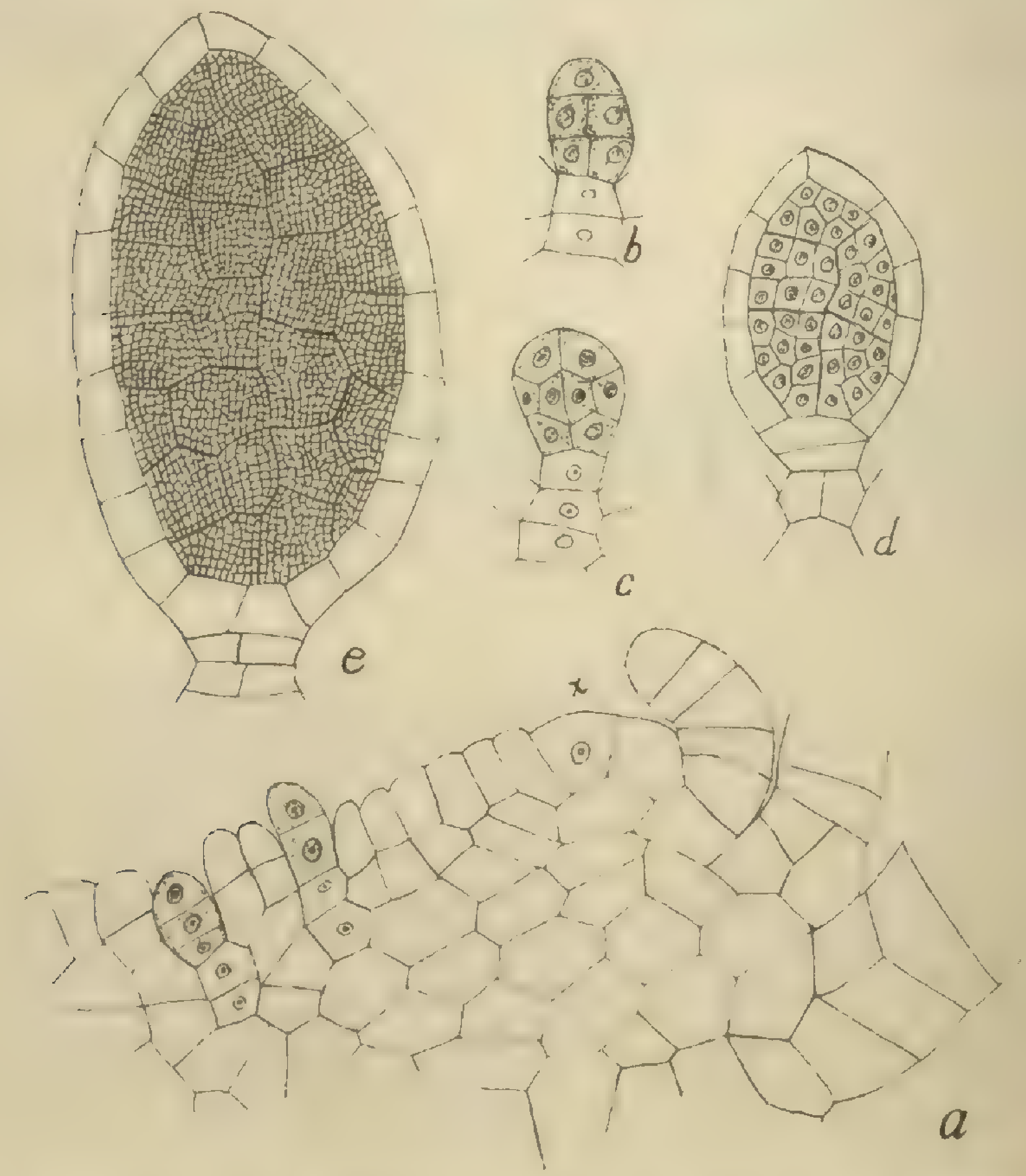

FIG. 2.—Antheridia. *a*, longitudinal section of apex of thallus with two antheridia; *x*, apical cell. × 600. *b*, *c*, *d*, successive stages of young anther × 600. *e*, full-grown antheridium. × 480.

antheridial cell, when it can first be distinguished from the initi cells around it, is a little larger and stains more deeply tha these cells. The first division (*a*, *fig*. 2) is transverse an divides the primary cell into the stalk or pedicel cell and the antheridial cell proper. The antheridial cell thus formed is then divided into three cells (*a*, *fig. 2*) by two transverse divisions. The next two divisions are longitudinal medial and are at right angles to each other, so that each of the three original cells is divided into four. In many cases, however, only the two lower cells are divided in this way (*b*, *fig. 2*), the top cell remaining undivided for a longer time. The four cells formed from the central cell by these longitudinal divisions are each again divided longitudinally, thus separating the sperm cells from those that go to make up a portion of the antheridial wall (*c*, *fig. 2*). The upper and lower cells take no part in forming the sperm cells, but form, respectively, the upper and lower portions of the antheridial wall. The remainder of the development does not differ materially from that of *Fimbriaria Californica* as described by Campbell.[4] The top of the antheridial wall is not prolonged, however, as in that species, but is only a single row of cells, as in Marchantia. In fact, the full-grown antheridium (*c*, *fig. 2*) resembles very closely that of Marchantia.

The sporogonial receptacle, or carpocephalum, is of Leitgeb's "Compositæ" type.[5] The apical cell of the thallus forms the growing point of the receptacle, but instead of remaining a single cell it divides into two cells. Each of these again divides in like manner. Finally one of the four cells thus formed divides into two, so that there are five growing points. In some cases this last division does not take place, so that there are only four growing points. Five is the usual number, however, for I found only two specimens out of the great number which I examined that had only four growing points. This branch system is somewhat emphasized in the half-grown receptacles, for, at this time, the lobes between the growing points are more developed than the rest, so that the underside of the receptacle has five quite prominent projections or folds. As the receptacle develops, however, these disappear.

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[4] Campbell, D. H.: Mosses and Ferns 50, 51. 1895.

[5] Leitgeb: Untersuchungen über die Lebermoose 6: 32.

The dorsal growth of the receptacle is excessive, while th ventral growth is limited to a few layers of cells. Consequent the apical cells (*a*, *fig. 3*) lie very close to the stalk. Th lacunæ or airchambers are for the most part confined to a sin layer. They are extremely large, however, and are separat

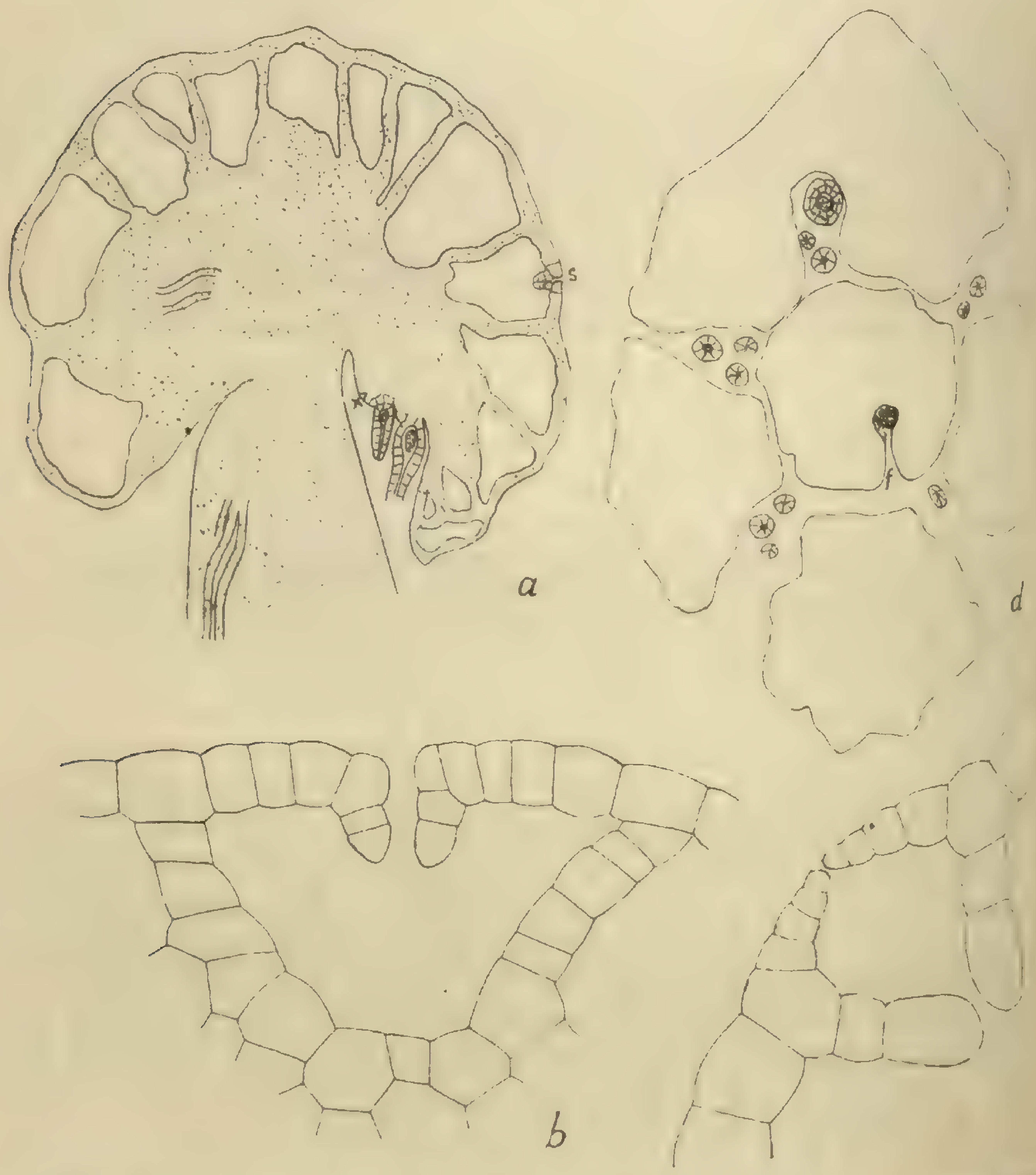

FIG. 3.—*a*, longitudinal section of sporogonial receptacle; *x*, apical cel stoma. × 80. *b*, *c*, longitudinal section of stoma. × 600. *d*, transverse section receptacle; *f*, furrow of peduncle. × 80.

from one another by a single row of cells. Each air-chamber is connected with the exterior by means of well-develope stomata. These peculiar breathing pores are present in sever of the Marchantiaceæ. They are almost cylindrical, and are composed of several rows of cells, which are formed from the original guard cells. These, instead of remaining single, divide by means of inclined walls into several cells. Generally four cells are cut off on the upper side (*b*, *c*, *fig. 3*), and two on the lower side of each original guard cell.

Stephani, *loc. cit.*, reports that there are two furrows on the ventral side of the stalk or peduncle of the receptacle, but I was unable to find such to be the case. In all the specimens examined there was only one. Mr. Howe,[6] also, reports only one furrow in the specimens examined by him. Seen in cross-section (*d*, *fig. 3*) it resembles very closely that of Fimbriaria and Duvalia as figured by Leitgeb.[7] The root hairs, all of which are tuberculate, are found in this furrow. At the base of the receptacle these branch, one branch going to each lobe between the growing points.

The archegonia, although they are on the underside of the receptacle, are in reality on the dorsal side, for they are formed acropetally just back of each apical cell. Hence there are five rows or groups of archegonia (*d*, *fig. 3*). In each of these rows there are usually three or four archegonia.

The development of the archegonium corresponds very closely with that of other Marchantiaceæ. The primary cell becomes much larger than the neighboring cells, and the cell contents becomes much denser, so that it stains very deeply. The first division is transverse. The outer cell forms the archegonium and the inner the stalk. Strasburger[8] states that in Marchantia this outer cell is again divided by a wall parallel to the first, and that the lower cell of the two thus formed forms the foot of the archegonium. Janczewski[9] describes the same thing in *Preissia commutata*. This second division does not take place in Cryptomitrium (*a*, *fig. 4*). Campbell[10] also states that it does n occur in Targionia nor in *Fimbriaria Californica*. The remainder of the development does not differ materially from that of typical archegonium of any of the Marchantiaceæ, and it need not be repeated here.

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[6] HOWE, M. A.: Erythea 5: 87, 88. 1897.

[7] LEITGEB: Untersuchungen über die Lebermoose 6: *pl. 4*, *figs. 9, 20*.

[8] STRASBURGER: Jahrb. f. wiss. Bot. 7: 416.

[9] JANCZEWSKI: Bot. Zeit.—: 418. 1872.

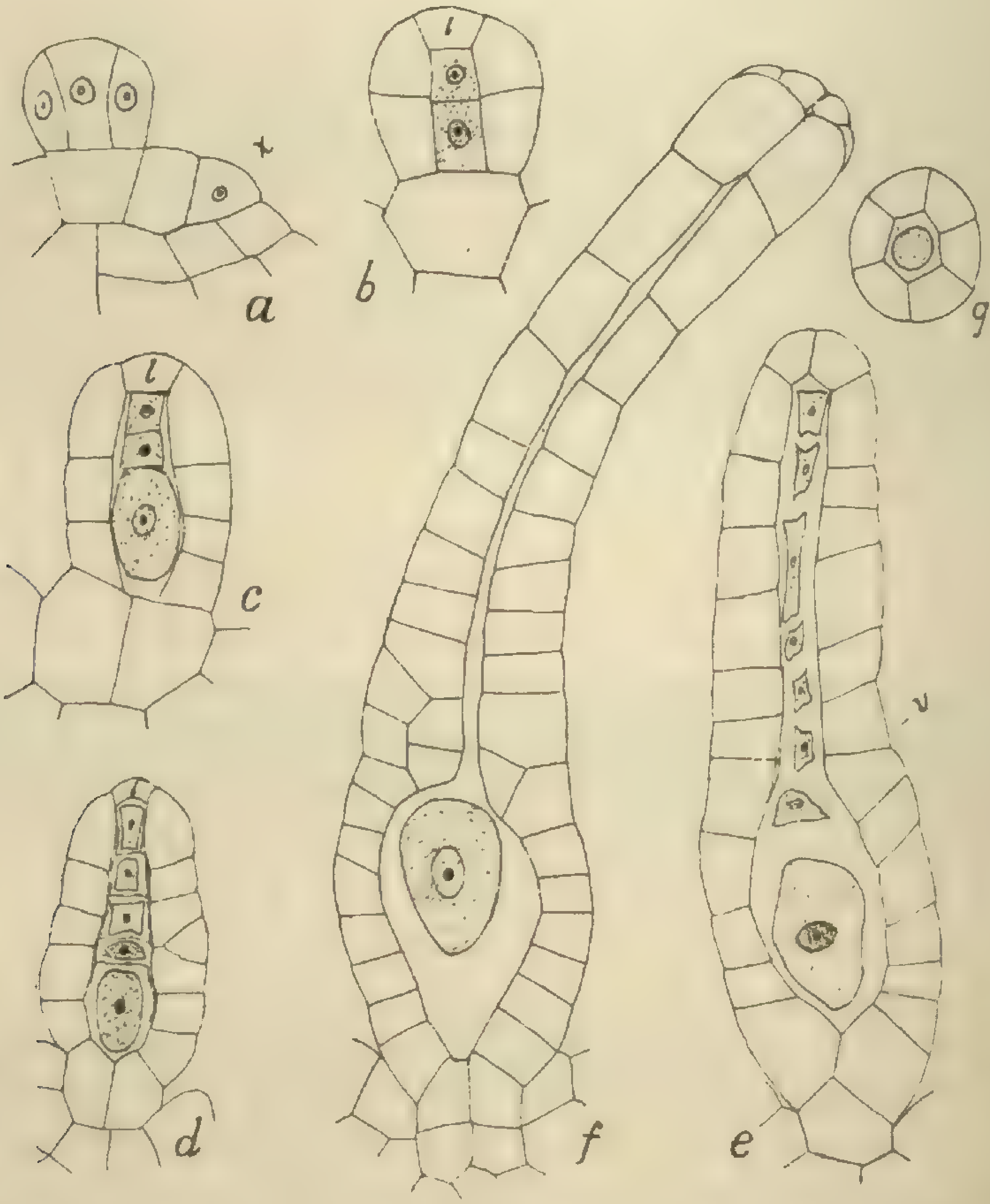

FIG. 4.—Archegonia. *a*, *b*, *c*, *d*, *e*, *f*, longitudinal sections of successive sta *x*, apical cell; *l*, *l*, *l*, cover cell; *v*, ventral canal cell; *g*, transverse section of nec an archegonium about the age of *e*. *a*, *b*, *c*, × 600; *d*, *e*, × 480; *f*, *g*, × 300.

The cover cell, which in other forms studied[11] divides in four cells immediately after the neck has been separated fr the venter, remains undivided for a considerably longer time this species (*b*, *c*, *d*, *l*, *fig. 4*). Unfortunately I was unable determine just when this division took place. Several archego were obtained at the age of the one represented in *d*, *fig. 4*, in every case the cover cell still remained undivided. The stages in which the division had taken place (*e*, *fig. 4*) were too old to determine with any degree of accuracy when this division occurred, and it is to be regretted that no intermediate stages were obtained.

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[10] CAMPBELL: *op. cit.* 52. 1895.

[11] CAMPBELL: *op. cit.* 30. 1895.

Gayet,[12] in a recent article, which has been reviewed by Campbell,[13] states that the archegonia of the Hepaticæ have a distinct apical growth, the same as in the Musci. His conclusions, which are contrary to those of Janczewski,[14] Campbell, and others, are not confirmed by my own observations. While I did not make a very careful study of this point, I could find nothing that indicated an apical cell. The cover cell, which Gayet claims to be the apical cell, does not have the appearance of one. It is much smaller than the upper cells of the neck, and in no way do these cells look as though they had been cut off from it. The fact that the cover cell remains undivided for a considerable time in this species might favor the idea of apical growth, were it not for the fact that the cover cell in this species is even smaller than in other species that have been studied (*d*, *fig. 4*). It looks as if it were lying dormant and not as if it were an active apical cell.

The usual number of neck canal cells is eight, as in the other Marchantiaceæ. In some cases, however (*f*, *fig. 4*), only seven are present.

THE SPOROPHYTE.

Owing to the fact that the embryo in nearly every case lies almost parallel with the stalk, its development has been comparatively easy to make out. One is at once struck with the marked similarity it shows to the embryo of Targionia.[15]

Soon after fertilization the egg cell enlarges to nearly twice its original size. The first division is transverse and divides the enlarged cell into two almost equal cells (*a*, *fig. 5*). The next division is longitudinal (*b*, *fig*. 5). This is followed by another longitudinal division which is at right angles to it. Each of the eight cells thus formed is then divided into two slightly unequal cells by a longitudinal division (*e*, *fig*. 5). The first longitudinal wall is often inclined so that the top cell (*c*, *fig*. 5) resembles very much a two-sided apical cell. The remaining divisions are

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[12] GAYET: Ann. des. Sci. Nat. Bot. VIII. —: ——. 1897.

[13] CAMPBELL: BOT. GAZ. **26**: 428–431. 1897.

[14] JANCZEWSKI: *loc. cit.*

[15] CAMPBELL: *op. cit.* 60, 61. 1895.

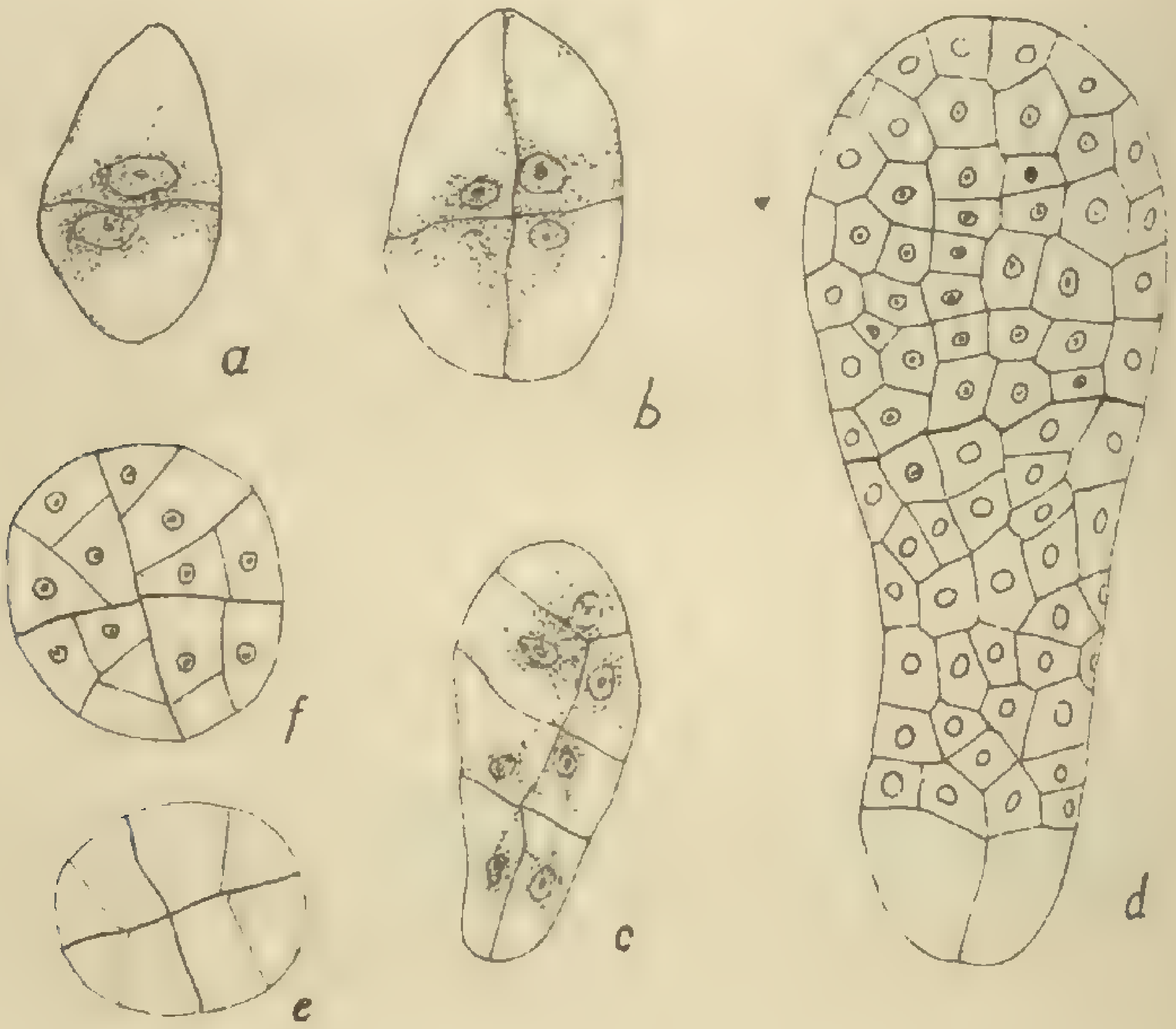

FIG. 5.—Embryo. *a*, *b*, very young stages in longitudinal section. × 600. *c*, somewhat older. × 480. *d*, longitudinal section of still older stage. × 300. *e*, transverse sections. × 480.

very irregular and are difficult to follow. The young embryo takes on a more and more elongated form. Finally, the central portion almost ceases to grow, so that the embryo becomes dumb-bell-shaped (*a*, *fig*. 6). The upper portion is to form the archesporium, and at about this time, or even earlier (*d*, *fig*. 5) a definite row of cells, which becomes the capsule wall, formed around the outside. Usually, the first transverse division marks the separation of the capsule and the foot, but in a few cases (*d*, *fig*. 5) this cell remained undivided after the first tw longitudinal divisions, so that at the base of the embryo we four large cells.

Soon after the capsule wall is formed the archesporial cells can easily be distinguished, for their protoplasm becomes denser and both it and the cell walls, which become very gelatinous, stain deeply (*a*, *b*, *fig.* 6). These gelatinous walls soon dissolve, so that the archesporial cells are set free. Two sorts of cells can easily be made out at this time. The one, the spore mother cells, are almost spherical. Their nuclei are large and distinct (*e*, *fig.* 6), and are surrounded by closely reticulated protoplasm. The other, the young elater cells (*d*, *fig.* 6), are elongated. Their nuclei, though quite distinct, are much smaller than those of the spore-mother-cells.

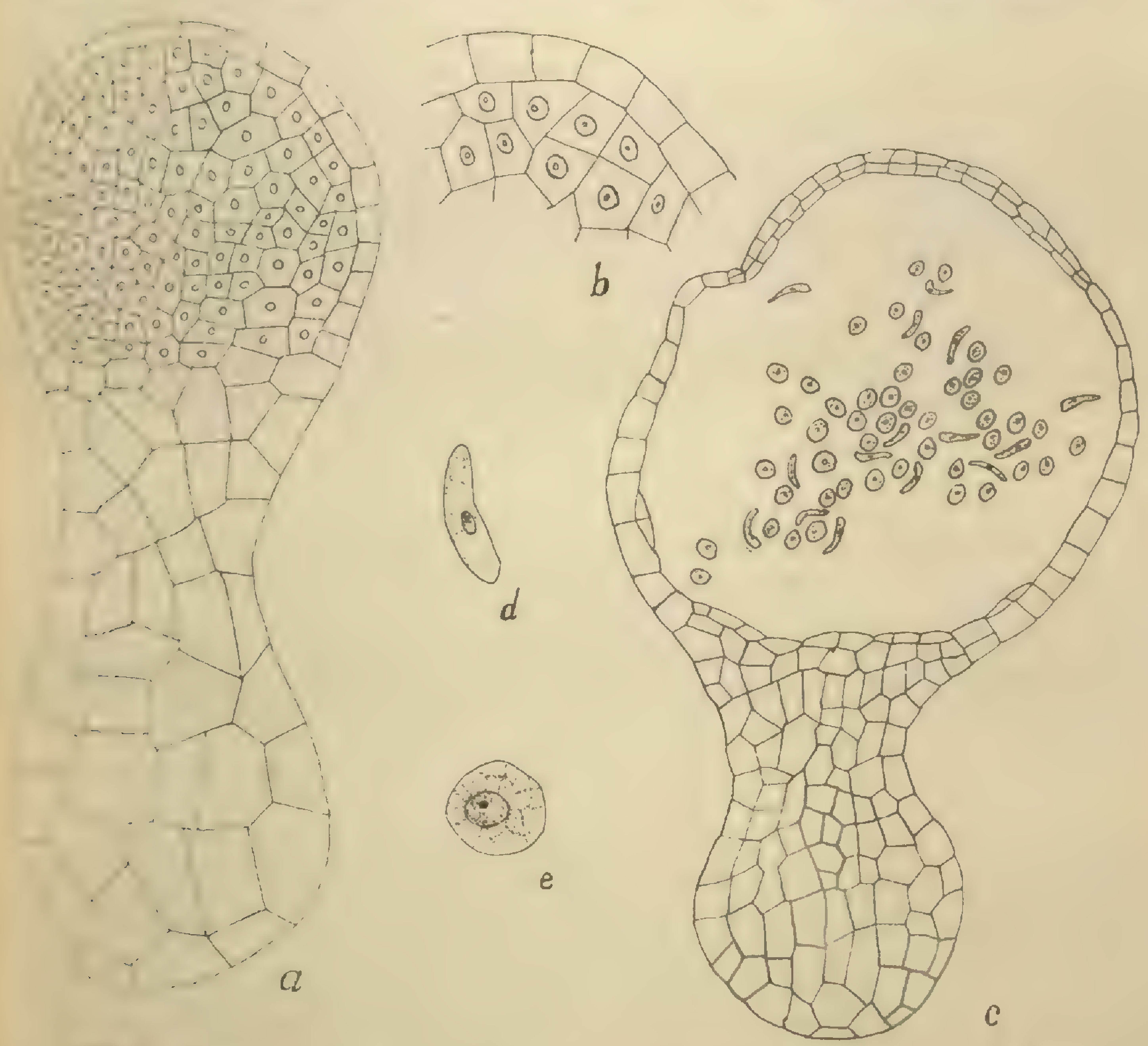

FIG. 6.—Embryo. *a*, longitudinal section of half-grown embryo. × 300. *b*, portion of transverse section showing capsule wall and archesporial cells. × 480. *c*, longitudinal section of a nearly mature sporogonium, showing spore-mother-cells and young elaters; *o*, operculum. × 150. *d*, young elater. × 600. *e*, spore-mother-cells. × 500.

The foot of the sporogonium (*c*, *fig.* 6) is not so well developed as in Targionia, being not more than one third as large as the capsule, which is large and globular. At maturity the capsule is regularly dehiscent at its apex by an operculum. This operculum, as stated by Howe (*loc. cit.*), is composed of two rows of cells (*o*, *fig.* 6), while the remainder of the capsule wall is, for the most part, only one cell thick. Near its base, however, an apparently continuous ring, composed of one or two rows of very small cells occurred in all the specimens examined.

The ripe spores germinate very slowly. During the months of October, November, and December, several cultures were made of spores which had ripened in the previous April, and in no case did they germinate until eighteen or twenty days after they were sown. Their germination and manner of growth correspond very closely to that of Targionia.

SUMMARY.

In comparing *Cryptomitrium tenerum* with the other Marchantiaceæ, I found, as Stephani claimed, that it was undoubtedly very closely related to Duvalia. I had no specimens of Duvalia, however, and was dependent upon Leitgeb's[16] description. Both genera are monœcious. Both have the same minute stomata surrounded by seven or eight very symmetrically arranged guard cells. Stephani states that Cryptomitrium has two furrows in the peduncle, while Duvalia has only one; but only one furrow was present in the specimens I examined, so that neither this difference between the genera, nor the difference in number of guard cells that Stephani described, exists.

The receptacle of Duvalia is nearly spherical, while that of Cryptomitrium is disk shape. In other respects the receptacles resemble each other very much externally; but in the development of the receptacles there is a great difference. Duvalia, according to Leitgeb's account, belongs to the type of the Marchantiaceæ, which has the growing point of the receptacle at the forward margin. In Cryptomitrium the receptacle is a branch system, such as Leitgeb attributes to Marchantia and one or two other genera. This fact alone would be enough to outweigh all the minor external characters referred to, were it not for the fact that, although Leitgeb puts Fimbriaria in the same type as Duvalia, Campbell (*op. cit.*) found that the receptacle of *Fimbriaria Californica* belongs to the "Compositæ," or branching type.

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[16] LEITGEB: *op. cit.* 87–90.

While one hesitates to criticise the classical work of Leitgeb, the quality and accuracy of which is for the most part remarkable, it does not seem reasonable that plants resembling each other as closely as Cryptomitrium and Duvalia should differ so much in respect to the growth of their receptacles, to say nothing of the fact that species of the same genus, Fimbriaria, should also have this difference. It would seem more probable that Leitgeb was mistaken. Probably if one should carefully examine Duvalia and also the species of Fimbriaria which were studied by Leitgeb, it would be found that these too have as many growing points as there are groups of archegonia. Should this not be the case the apparent close relation between Duvalia and Cryptomitrium would be only apparent, and the latter would then, perhaps, have to be considered more nearly related to Marchantia, and *Fimbriaria Californica* could then no longer be considered as a Fimbriaria, for the difference in the two kinds of receptacles is too great to occur within the same genus.

Leland Stanford Jr. University.

BRIEFER ARTICLES.

NOTES OF TRAVEL. I.

VENEZUELA.

THE aim of the expedition with which the writer is connected, was planned by Mr. Barbour Lathrop, of Chicago, and carried out at his own expense, has forbidden any exhaustive research into the botanical resources of South American countries. It has permitted rapid comparisons, however, and it is these comparative sketches which it is believed will interest American botanists.

The first approach to a great continent, if it has thousands of square miles of unexplored territory in it, as South America has, is always impressive. La Guayra, the principal port of Venezuela, satisfies one's preconceived notions of tropical luxuriance of vegetation. The steep mountains behind the town shut it in like a green wall, and the low hanging clouds and dark rainy valleys, into one of which the famous railroad to Caracas disappears, are characteristically tropical. By characteristically tropical the writer may give a wrong impression, since what could be characterized as tropical in one region might not be true of another. The xerophytes are as abundant in the tropics as in temperate regions, although in the popular mind they are not characteristic of the tropics. Venezuela landscapes show a larger proportion of xerophytes than I had expected to see, and a ten minutes' tram ride to the small bathing place of Moquendo gave me a good opportunity of seeing the characteristic cactus vegetation of the coast. Almost barren patches of reddish-brown soil and frequent signs of prairie fires on the hillside surprise one, while the tufted grasses, agaves, and cacti give the whole a decidedly arid look. The climate of La Guayra is a dangerous one for foreigners, as the malarial fevers there are very severe. We were informed, however, by intelligent English people living in Caracas that the latter are no more severe than those of Caracas itself. From my friend's most uncomfortable experience it is evident that the capital has a serious form of malarial fever, and great care must be exercised to avoid exposure after sunset.

[illegible]

The La Guayra and Caracas railway has some of the most picturesque scenery in the world. Twenty-three miles of track are necessary to cover seven as the crow flies, and the curves and zigzags along the coast give glimpses of great grandeur. The disappointing part of the landscape lies in barren soil and unmistakable signs of aridity. Curious cereuses and acacias or Prosopis, and a gigantic species of Asclepias with flowers three times the size of our *A. Cornuti* attract one's attention, while the fine-leaved forest trees in the valleys give the landscape a much more northern aspect than would be expected. Two views strike the traveler most favorably; one from a curve in the road which overlooks the coast, where, spread out below, are plantations of sugar cane and banana, fringed with the most graceful of cocoanut palms that stand out like dark green plumes against the white surf; and the second, some distance nearer Caracas, where the road crosses a ravine which drops into a narrow valley, 1500 feet deep and completely clothed with forest.

The vegetation effect, while most impressive, is not truly luxuriant, and unmistakable signs of aridity are everywhere present. Curious arid ridges and isolated peaks along the sides of scantily covered valleys give the impression of poor soil and rapid erosion. There is a rumor that these arid patches were once wooded, but that injudicious removal, constant forest fires, and later prairie fires have denuded them. Dr. Ernst, the Venezuelan botanical authority in Caracas, does not believe this. He declares they have been barren from prehistoric times.

Caracas lies 2632 feet above the sea, surrounded by barren hillsides whose summits are covered with dense forest.

There is very little of botanical interest in Caracas itself. A few interesting private gardens lie across the small stream which flows through the town. One in particular contained a number of curious cultivated plants. In it was the most remarkable fountain I have ever seen. From a hideous cement imitation of a boa constrictor spouted a small stream of water. Around, on little artificial islands, were statues of storks in the act of swallowing. Growing on these islands were large guava trees, oranges in fruit, and tufts of Egyptian papyrus, while for a border to the basin a row of blossoming strawberry plants and Chinese hibiscus bushes had been planted.

The coffee estate of Señor R. Dalla Costa Mosquera, one of the few within the city limits, is well worth a visit and has in it a magnificent avenue of St. Domingo mahogany trees. It illustrates the coffee culture of Venezuela very well, which is in marked contrast to that of Brazil by its employment of shade trees. These cast a relatively deep shade over the whole plantation, and gave the impression of a thickly planted grove. In Brazil no shade trees are employed, and in Ceylon and Java they are planted very sparsely among the coffee trees. I seriously questioned the advisability of such heavy shading and was informed simply that it was considered advantageous. Some of the best coffee in the world is grown in Venezuela, but very little of it reaches the American market because the latter demands principally the cheaper Brazilian sorts. On the best Venezuelan estates the method of pulping the coffee berry before drying is in use, while the majority of Brazilian coffee growers still cling to the old method of drying the berry first and removing the dried pulp afterward. There are large Brazilian estates where this method has been given up and the best machinery is in use.

Nothing was seen in Caracas of the cocoa industry, although some of the finest cocoa in the world is grown along the Venezuelan seacoast and in the interior about Maracaibo.

In every city one of the most interesting places for a botanist is the market, and in Caracas it is characterized by an extraordinary show of flowers. Tuberoses, white double violets, delicate purple irises, Easter lilies, and curious bouquets made up of double columbines, marigolds and lilies surrounded with tissue-paper lace were most in favor at carnival time. Curious pear-shaped, thick-skinned shaddocks which are used for preserve making; long, chocolate-brown, melon-like squashes, with orange-yellow flesh; immense green watermelons with squash-like meat, showing how careless the growers are about the interbreeding of their squashes and melons; bright yellow "curumas" with orange-colored, mealy flesh, related to the curcumas of Peru, which are used in the manufacture of a favorite ice; peaches and apples of inferior quality grown in the mountains of the interior, together with the usual number of vegetables of quite inferior varieties compose the piles of produce on the well regulated stalls. This part of the market is much more appetizing than the other sections where the most disgusting looking strings of salted meat are hung for sale.

There is in Caracas a National Society of Agriculture which aims to diffuse intelligence regarding the culture of agricultural products.

to teach the use of fertilizers and soiling crops, etc. But it is governmental, and when that is said of almost any concern in South America, it means that it is subject to rapid political changes.

The courtesy of Mr. Alamo, Assistant Secretary of Agriculture and Mr. Romero, secretary of the society, could scarcely have been greater.

To any foreign scientist, Dr. A. Ernst, the professor of botany in the University, is an invaluable acquaintance. His long experience with Venezuelan conditions and his fund of information on the botanical resources of the country are the result of a wide acquaintance and numerous expeditions into the interior. His advanced years make it impossible for him to continue his work as a collector, but his vigorous mind and excellent memory make his suggestions most valuable to a traveling scientist.

As a young German botanist he came to Venezuela on a collecting trip and was invited by the government to remain. Revolution has followed revolution, but, through his refusal to meddle in the politics of the country in the first place, and his undeniable ability as a scientist in the second, he has kept the position he now holds as one of the most profound scientists and highly esteemed citizens of the republic. His years of labor in an attempt to educate Venezuelan youth to an appreciation of botany have not, it must be regretted, left him hopeful of the final outcome, and from what the writer could learn there is nothing to encourage foreign scientists to engage in governmental work in Venezuela.

Two railways lead from Caracas into the interior, one English, the other German; neither, however, takes the traveler anywhere near the most interesting region of the Sierra Nevada, which lies, by mule or horseback, some four to five days travel from Caracas. In this Sierra Nevada range are snow-capped peaks and a vegetation that is said to be most luxuriant and peculiar. For the botanical explorer this interior mountain region will prove rich in new forms. Mr. André of Trinidad, a well-known orchid collector, informs me, however, that orchids are more abundant near the coast in the neighborhood of Cumana. In order to explore these interior regions the traveler must have at least a fair knowledge of the Spanish language and should arrange his baggage in square leather boxes not over two feet in largest dimension, suitable for donkey or mule transport. The food obtainable will be of the very poorest quality; and, as the *peones* live in huts of most unsanitary character, great care will be necessary to avoid contracting the numerous diseases associated with such conditions.

Caracas is not a favorable place from which to explore the resources of Venezuela. It lies too far from the most interesting portions of the country. The Orinoco can be ascended better from Trinidad, and the Sierra Nevada requires an expedition on muleback to reach it. From descriptions given by travelers on the Orinoco and its branches, the dangers from fever in the forest regions of Venezuela are very great and anyone undertaking their exploration risks his life. Mr. E. André, whose travels into the interior have been as extensive as any in recent years, said he would not think of taking with him any person who had not lived at least two years in the tropics and become acclimated as far as possible to conditions similar to those in Venezuela.—DAVID G. FAIRCHILD, *U. S. Department of Agriculture.*

SOME SPECIES OF TETRANEURIS AND ITS ALLIES.

WE sometimes hear the statement that the difficulties for the systematic botanist are being multiplied by the breaking up of so many of the old genera and the creation of new species from former aggregates, but practical experience shows, it seems to me, that segregation, when based on describable characters, certainly simplifies. The replacing of the untenable *Actinella* by *Tetraneuris*, *Rydbergia*, and *Picradenia* (Pitt. 3 : 265), is a case in point.

The reduction of several good species to one (an aggregate) makes necessary a description so general that the amateur in the field has no difficulty in placing the most aberrant form until he collects a suite of specimens clearly unlike. In the past, reduction of species has often occurred because certain ones were rare and hence not well represented in the herbaria, but it seems unfair to eliminate a species simply because it exists in a locality not easily accessible or rarely visited.

Being located in the center of distribution of *Tetraneuris* and its allies, I became interested in the group. The following notes and descriptions are offered as supplementary to Dr. Greene's valuable paper cited above.

TETRANEURIS ACAULIS (Pursh) Greene, Pitt. **3**: 265. 1898.

Galardia acaulis Pursh, Fl. **2**: 743. 1814. *Actinella acaulis* Nutt. T. & G. Fl. **2**: 381. 1842, etc.

As limited by the earlier writers this is a variable but a recognizable species. When many of the following were incorporated, the difficulties of the field botanist were multiplied several fold in respect to this species.

TETRANEURIS ACAULIS **cæspitosa**, n. var.—Strongly matted, depressed-spreading, the numerous branches of the caudex much thickened by the imbricated leaf-bases: leaves very numerous and crowded, densely silky-lanate as are also the scapes and involucre: heads nearly sessile or on scapes 3–6^{cm} long.

That specimens of this variety exist in some herbaria as *T. acaulis* is possible, though in the large series in the Herb. Mo. Bot. Garden none were found. Its matted habit, silky-lanate leaves and very short scapes easily separate it. It occurs sparingly on sandy ridges in the foothills. Laramie hills nos. 1890 and 4314 represent it.

Tetraneuris simplex, n. sp.—Tap root vertical, short, comparatively small with few or many secondary roots: caudex short, consisting of one or more thick crowns which are densely covered with brown dead leaf-bases: leaves appressed-pubescent (not silky), nearly naked in the axils or sparsely long-hairy, glabrate in age when the fine punctation becomes evident, crowded on the crowns, ascending or erect, linear spatulate, tapering only slightly to the margined base, subacute, 4–7^{cm} long: scapes simple, single from the crowns, 15–25^{cm} high, slender, erect, lightly pubescent below, becoming silky or lanate above and on the involucre: head large, 2.5–4^{cm} across; rays with a broad ligule (5–8^{mm}): akene pubescent.

To include this with *T. acaulis* is not conducive to clearness. Dr. Greene (l. c.) has separated some of the other well marked species and this seems to me to be as good as the best of them. I have had this and *T. acaulis* under observation for several years, and there is not the slightest possibility of confounding the two in the field. *T. acaulis* is always cespitose, often in considerable mats, the scapes are shorter, the heads smaller, and the rays narrower than in *T. simplex*. The leaves of the latter are comparatively glabrate from the first, strongly in contrast to the silky or even lanate pubescence of the other.

That collectors have never been inclined to call this *T. acaulis* is shown by the fact that specimens of it occur in the herbaria just as often ticketed *T. scaposa* or *T. scaposa linearis*, though to these it is not so closely related. Whether *T. acaulis* or *T. simplex* is the original *Galardia acaulis* is difficult to determine, but the term "pilosa" in the original description, and the agreement in Nutt. Gen. 173 and T. & G. Fl. that the leaves are "sericeous (silky) villous," and that the plants are aggregated in dense tufts, sugges the separation that is now proposed.

Besides a large series of plants from near Laramie, specimens of T. *simplex* have been examined as follows: T. A. Williams, Pine Ridge, Ne J. Schenck, Neb., 1893; H. J. Webber, Pine Ridge, Neb., 1889; Hen Engelmann, North Fork of the Platte, 1858; A. S. Hitchcock, Kan., 18 no. 289; C. H. Thompson, Kan., 1893, no. 169; Capt. Bryan's Expedit Lower Pole Creek, Wyo., 1858; R. S. Williams, Great Falls, Mont., 18 no. 82; G. E. Osterhout, Livermore, Colo., 1898; M. E. Jones, Cheyen Cañon, Colo., 1878; Hall & Harbour, no. 275.

Tetraneuris incana, n. sp.—Root rather slender, simple or branched caudex simple or few branched, the crowns enlarged by a den covering of the broadly expanded bases of the petioles whi are more or less involved in white, hirsute wool: leaves crowded the crowns, silvery-white with an appressed pubescence, linear-obl ceolate, 2–4^{cm} long: scapes naked, single from the crowns, slend curved-ascending, 1–2^{dm} high, the fine silvery pubescence sligh spreading: involucre silvery-silky, bracts few, shorter than the 1^{cm} h disk, the outer oblong, obtuse, the inner spatulate, scarious margine rays few, the ligule as long as the disk: disk corollas sprinkled w resinous globules and toward the summit strongly thickened by a den penicillate, glandular beard: pappus scales oblong, aristate: aken slender, nearly as long as the corolla, pubescent.

This rare species is strongly marked in its close, silvery pubescense, nearly simple caudex, its silvery involucre, and its dense coat of glandular ha on the corollas. The only collections of it at hand are no. 393, Platte h near Fairbanks, July 11, 1894; no. 5006 (type number), by Mr. Elias Ne Wallace creek, July 30, 1898; and a specimen by Mrs. Muth, Lewis & Cla co., Mont. Its habitat is white clay ridges among the barren hills.

TETRANEURIS TORREYANA (Nutt.) Greene, l. c.

Actinella Torreyana Nutt., Trans. Am. Phil. Soc. 7: 379. 1841.

A strong species of the central-eastern Rocky mountains: in sev eral forms, but always tufted, strongly punctate and nearly glab except on the caudex; somewhat variable as to the width and rigi of the leaves. My no. 4810, from the Platte hills near Fort Ste June 18, 1898, are nearly typical; nos. 4571 and 4747, June 1898, fr the Tertiary clays of south-central Wyoming have broader, laxer lea than the original description permits. A form represented by no. 4327 and some earlier collections, from the limestone ledges of the Laramie hills, is strongly matted and has the branches of the caudex enormously thickened and protected by the densely lanate leaf-bases. Add to this its large root, broad green leaves, and the copious secretion of its punctate glands, and it might well stand as var. *glandulosa.* It is in this species that the salient character of the genus (4-nerved ligules) often fails; 5–8 nerves are not infrequent.

Tetraneuris Mancosensis, n. sp.—Tufted, with woody root and multicipital caudex, the short thickened crowns clothed with the expanded, membranous, lanate leaf-bases: leaves glabrous, 4–8^{cm} long, crowded on the crowns, linear or linear-oblanceolate, acute or cuspidate, rather minutely punctate: stems few to several, bearing a few (usually 2) distant leaves, 2^{dm} in length (including the long monocephalous peduncle): heads large, disk about 1^{cm} high; involucre silky-lanate, the bracts in two or three rows, the inner oblong or somewhat expanded upwards by the scarious margins: paleæ of the pappus oblong-elliptic with an acumination as long as the body proper, equaling the disk corollas: ligules of the rays 15–18^{mm} long, 6–8^{mm} broad.

Collected by Professor C. S. Crandall, Mancos, Colo., June 29, 1898, and distributed as *Actinella scaposa linearis* Nutt. It is in fact, however, much nearer to *T. Torreyana*, from which its slenderer, longer, and less conspicuously punctate leaves, its nearly glabrous two- or three-leaved stems, its long-peduncled heads, and long pappus paleæ at once separate it.

TETRANEURIS LANATA (Nutt.) Greene, l. c.
Actinella lanata Nutt. l. c.

A rare plant of the arid interior, on dry ridges on the high plains. Only the following collections of it have been secured by the writer: 3265 and 4726 from Green river, in 1897 and 1898 respectively; 4607 from Ft. Bridger, June 9, 1898 are young specimens, but probably the same. More or less of the wool is permanent even on the leaves, though these show some punctation on the glabrate areas.

RYDBERGIA GRANDIFLORA (T. & G.) Greene, l. c.
Actinella grandiflora T. & G., Journ. Bost. Soc. Nat. Hist. **5**: 110. 1847.

This occurs in abundance in the alpine regions of all our mountain ranges.

PICRADENIA RICHARDSONII Hook., Fl. **1**: 317. *pl. 108.* 1833.—That authentic specimens of this occur in this range is possible, but it seems quite certain that the wide range attributed to this species in Gra Synoptical Flora is due to other species being included. With erection of *P. floribunda* (Gray) Greene and *P. canescens* (Eaton) Gree into species the area covered has also been segregated. But even the establishment of the two following species some Rocky moun forms, such as my no. ——, are still left to represent the original species.

PICRADENIA LIGULÆFLORA Aven Nelson, Bull. Torr. Bot. Club 25 378. 1898.

This species is proving to be far more common than was at fi suspected. As to habitat see notes on the following species.

Picradenia macrantha, n. sp.—Caudex branched, each branch mounted by a few to several crowns; crowns clothed with the leaf-bases: stems single from each crown, erect, fascicled, spars pubescent, somewhat striate, about 15^{cm} high: leaves glabrate, not spicuously punctate, rather numerous on both crowns and ste slender petioled, the blade variously parted into linear divisions, of them pedately trifid, some of the stem leaves pinnately parted twice trifid, the uppermost sometimes simple: heads large, pedun one to five on each stem (generally two or three): involucre sh than the disk flowers, outer bracts lanceolate, nearly glabrous, un for half their length, 6^{mm} long; inner oblong, acute, scarious margin rays 6–10, chrome yellow, the ligule $15–18^{mm}$ long and one third broad: pappus scales 5–7, lanceolate, shorter than the corolla.

Allied to *P. ligulæflora*, from which its larger size, conspicuous longer peduncles, fewer and less resinous heads serve to separate it. habitat of this is open, stony slopes in the mountains or hills, while *P. lig* *flora* occurs on dry, clayey, alkaline ridges or flats on the open plains. number 4830, Fort Steele, June 18, 1898. What seems to be the same is no. 1688, Centennial hills, Aug., 1895; also South Park, Colo., 18 Marcus E. Jones.—AVEN NELSON, *University of Wyoming.*

PYCNANTHEMUM VERTICILLATUM, A MISINTERPRET MINT.

MR. W. W. EGGLESTON has called my attention to a Pycn themum, abundant about Rutland, Vermont, which has been p by recent botanists with both *P. muticum* Pers. and *P. Torreyi* Ben but appears different from either of those species. In its the plant matches the original plate published by Michaux[1] with his description of *Brachystemum verticillatum.* Fortunately a portion of Michaux's type specimen is preserved in the Gray Herbarium, and a careful comparison with that leaves no doubt that Mr. Eggleston's plant is the same. Another Vermont specimen collected by Robbins, and a number of specimens from more southern states possess the same characters as the Michaux plant, and with it represent a species fairly distinct from either *P. muticum* or *P. Torreyi.*

Brachystemum verticillatum was described by Michaux from the mountains of Pennsylvania and Carolina. It was soon transferred to Pycnanthemum by Persoon,[2] who merely accepted Michaux's description without comment. Pursh in his *Flora*, took up the name, modifying the description slightly, but Pursh's plant is said by Bentham, in his monograph,[3] to be the same as *Pycnanthemum aristatum* Michx. In the same work[4] Bentham placed *P. verticillatum* under *P. lanceolatum* Pursh ; but subsequently, in De Candolle's *Prodromus*,[5] he reinstated it as a doubtful species. In the *Synoptical Flora* Dr. Gray treated the plant as a form of *P. muticum* Pers.

In 1891 Otto Kuntze[6] took up the obsolete[7] generic name *Koellia* Moench,[8] and transferred to it Michaux's *Brachystemum verticillatum*, though from his statement it is apparent that he meant *Brachystemum muticum*, and was merely following Dr. Gray in regarding that and *B. verticillatum* as the same. In 1894, in their list of the plants of southwestern Virginia, Dr. Small and Miss Vail treated the plants as different species, Dr. Britton[9] there transferring *Brachystemum muticum* Michx. to Koellia. *K. Torreyi* Kuntze (*Pycnanthemum Torreyi* Benth.) was also then recognized as a distinct species. In the *Botan-*

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[1] MICHX., Fl. **2** : 6. *pl. 31.*

[2] PERS. Syn. **2** : 128.

[3] BENTH. Lab. 328.

[4] BENTH. *l. c.* 330.

[5] DC. Prodr. **12** : 190.

[6] O. K. Rev. Gen. **2** : 520.

[7] There is little reason to suppose that the name *Koellia* will be generally accepted among botanists. Though that name is printed instead of Pycnanthemum in Engler and Prantl's *Natürlichen Pflanzenfamilien*, Professor Urban of Berlin writes that, not having been used within fifty years of the time of its original publication, it must be rejected by the "Berlin rule"; and that only through an unfortunate oversight was Pycnanthemum not taken up in its stead in the preparation of the *Supplement* to Engler and Prantl's work.

[8] MOENCH Meth. 407.

[9] BRITTON in Small and Vail, Mem. Torr. Bot. Cl. **4** : 145.

ical Club Check List,[10] however, *P. Torreyi* Benth. is reduced *verticillata*. Thus within three years this plant, which was obscure to the great monographer of the Labiatæ, under the in ing influence of a resuscitated generic name was combined wi least two other species: Otto Kuntze, following Dr. Gray, consid the plant a form of *Pycnanthemum muticum*; then it was treated species distinct from both *P. muticum* and *P. Torreyi;* then d the same year it was united with *P. Torreyi.*

As stated above, a study of the type of *Brachystemum verticil* Michx. shows that plant to be fairly distinct from either his *B. mu cum* or the later *Pycnanthemum Torreyi* Benth. The follow description and notes may help to distinguish this plant, and show points of similarity and of difference between it and those with w it has been associated.

PYCNANTHEMUM VERTICILLATUM Pers.—Stem shortly and rather closely pubescent especially above, the branches loosely subcorymbose: leaves ovate-lanceolate or lanceolate, entire or slightly toothed. sessile, mostly glabrous; the reduced upper ones subtending rather, dense terminal or verticillastrate glomerules, densely pubescent: bracts of the glomerules ovate-lanceolate with subulate more or less pubescent on the backs, and with ciliate margins: 5.5^{mm} long, with the 5 hispid lance-subulate teeth about equal: 7 or 8^{mm} long, the upper lip short-oblong or obovate, the lower the oblong middle lobe twice exceeding the lateral ones: sta included, scarcely 1^{mm} long.—Syn. **2**: 128; Benth. in DC. Prodr. 190; Gray, Am. Jour. Sc. **42**: 47. ***P. lanceolatum*** Benth. Lab. part, not Pursh. *P. muticum* Gray, Syn. Fl. **2**: 355, in part, not ***Brachystemum verticillatum*** Michx. Fl. **2**: 6. *pl. 31*. *Koellia cillata* Kuntze, Rev. Gen. **2**: 520 (as to synonym only); Small Vail, Mem. Torr. Bot. Cl. **4**: 146 (at least as to Farmer Mt. spec but not as to note, which apparently refers to *Pycnanthemum cli ioides* Gray); Britton, Mem. Torr. Bot. Cl. **5**: 280 (excluding *Pycnanthemum Torreyi* Benth.).—Specimens examined: Pennsy (*Michaux*); Colchester, Vermont (*Robbins*); Rutland, Royalton Clarendon Springs, Vermont (*W. W. Eggleston*); Billerica, Mass setts (*C. W. Jenks* and *C. W. Swan*); Dedham, Massachusetts *Alice Browne*); Providence, Rhode Island (*George Thurber*

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[10] Mem. Torr. Bot. Cl. **5**: 280.

York (*J. Carey*); Easton, Pennsylvania (*T. C. Porter*); Farmer Mt., Carroll co., Virginia (*John K. Small*).

Habitally *Pycnanthemum verticillatum* should rarely be confused with either *P. muticum* or *P. Torreyi*. *P. muticum* has broader ovate-serrate leaves, the upper distinctly whitened; the bracts of the glomerules are linear or lance-attenuate; and the long stamens are generally much exserted. Though in foliage *P. verticillatum* is approached by *P. muticum*, var. *pilosum* Gray, that form is readily distinguished by its characteristic pubescence and bracts.

From the somewhat similar *P. lanceolatum*, to which Bentham once referred it, *P. verticillatum* is distinguished by its broader leaves, the upper ones pubescent; by its more open inflorescence; by the longer more acuminate and less pubescent bracts of the glomerule; and by the sharper calyx-teeth and smaller corolla.

Some forms of *Pycnanthemum Torreyi* approach *P. verticillatum*, but usually that species may be readily told by its thinner narrowly lanceolate leaves, distinctly tapering at the base, the upper mostly glabrous; and by the narrower bracts of the glomerule. The stamens of the two plants present a striking difference. In *P. Torreyi* they are long exserted, equaling the upper lip of the corolla; while in *P. verticillatum* they are scarcely 1^{mm} long, and included within the throat of the corolla. It is possible that this is a case of heterostyly, which is rarely observed in *P. lanceolatum*, and that the two plants should be considered extreme forms of one species. From the facts, however, that this floral character, in all the specimens examined, is accompanied by definite characters of pubescence, leaf, and bracts; that *P. verticillatum* extends much further north than the narrow leaved *P. Torreyi*; and that, in one point or another, *P. verticillatum* approaches equally near to *P. lanceolatum*, *P. muticum*, and *P. Torreyi*, it seems more reasonable to treat it as a species. The specific characters in the genus Pycnanthemum are not so fixed as would be convenient for arbitrary diagnoses; but as species go in the group, *P. lanceolatum* and *P. linifolium*, *P. muticum* and *P. incanum*, for example, *P. verticillatum* seems worthy of recognition.—M. L. FERNALD, *Gray Herbarium, Cambridge, Mass.*

THREE NEW CHORIPETALAE FROM NORTH AMERIC AND MEXICO.

Silene rectiramea. — Cespitose perennial, 2 to 3^{dm} high: stems se eral from a multicipital caudex, covered at the base by the pale scari persistent scale-like ciliated bases of the earliest leaves, terete or ellip in section, slightly striate in a dried state, pubescent and more or le viscid especially above, sometimes simple to the inflorescence, some times branched from every node; lower internodes relatively shor often curved, the middle and upper elongated, 6 to 8^{cm} long, mu exceeding the leaves, remarkably straight; branches solitary or opp site at the nodes, diverging from the stem at a uniform angle of ab 45°; their internodes also elongated and very straight: leaves of t stem about five pairs; the lower ones, like the radical, oblanceola 2.4 to 4^{cm} long, 4 to 7^{mm} broad, the middle and upper lance-oblong linear, all acute, 1-nerved, obscurely pinnate-veined, minutely papil and pulverulent-puberulent under a strong lens: bracts lance-line often purplish, 7 to 9^{mm} long; bractlets similar, 2^{mm} long: flowe terminal on the divergent branches of and open flat-topped cyme, in weaker stems reduced to a terminal and one or two short-pedice lateral ones; calyx cylindric in anthesis, white and papery but vein with light purple, 9^{mm} long, 10-nerved; the nerves opposite the tee branching freely, intermediate ones subsimple; teeth orbicular w incurved margins: gynophore in anthesis 1.7, in fruit 2.5^{mm} long: pe 1 to 1.1^{cm} long, glabrous except externally at the very base; claws ulate, subauriculate at the summit, 3-veined; blade short, 2 to long, bifid a fourth of its length: stamens 10, equal: carpels 3: ca sule ovoid, 7^{mm} long, at maturity 1-celled to the very base: seeds re dish-brown, tubercles in few concentric series, those of the dorsal reg enlarged and forming a more or less definite crest.

Collected by Professor D. T. MacDougal about the Grand Cañon of Colorado in Arizona, altitude 2150^{m}, 28 June, 1898, no. 181. Type in he Gray.

This species stands near *S. verecunda* Watson, but differs in its e gated very straight branches and delicate papery calyx, which, although rowly cylindric to obovoid, shows no indication of the tightening or cons tion about the carpophore which is to be noticed in *S. verecunda*. Specimens *S. rectiramea* were distributed in Mr. MacDougal's interesting Arizona se but were determined only to the genus. Mr. A. A. Heller, who has charge of the identification of the sets, has courteously waived in fav author his right to characterize this species.

Arabis Crandallii. — Cespitose perennial, 3^{dm} high, pale green and hoary puberulent throughout, with minute stellate interplexed hairs: stems numerous (20 or more), slender, terete, from a loosely multicipital caudex: root single, vertical: radical leaves oblanceolate-spatulate, 1.5 to 1.8^{cm} long, 3 to 4^{mm} broad, entire, acutish, cuneate-attenuate at the base, concolorous, minutely stellate-tomentulose on both surfaces, 1-nerved; the cauline (about 8 on each stem) similar but shorter and more oblong, sessile by a subamplexicaul base: pedicels ascending or appressed, 5 to 6^{mm} long, slightly enlarged at the summit: sepals oblong, obtuse, stellate-puberulent, often purplish-tinged, 3^{mm} long: petals obovate, cuneate, white, roseate-tinged, twice as long as the calyx: pods erect, slender, subtorulose in dried specimens, 2.5 to 4^{cm} long, 1^{mm} broad, flattened; seeds (immature) uniseriate in each cell and nearly or quite as broad as the septum.

Collected by Professor C. S. Crandall at Cimmarron, Montrose co., Colorado, altitude 2100^{m}, 18 May, 1898, no. 6. Type in herb. Gray.

In habit this species most nearly approaches the Canadian *A. Macounii* Wats., from which, however, it is clearly distinguished by its fine stellate pubescence, shorter erect pods, and larger leaves.

Mimosa acapulcensis (Subg. *Eumimosa* Ser. *Sensitivæ*). — Shrub 1.5 to 3^{m} high: branches terete, subflexuous, covered with a yellowish-gray striate cortex and armed with scattered, firm, grayish-brown, laterally compressed, slightly recurved spines: leaves unijugate and pinnæ 3-foliolate in the manner of *M. sensitiva* (a diminutive fourth leaflet occasionally present); leaflets glabrous, glaucous, coriaceous, oblong, entire, cartilaginous-margined, acute to rounded at the apex, subcordate and very oblique at the base, oblong, 3 to 10^{cm} long, nearly half as broad; common petioles slender, wiry, 3 to 8^{cm} long; secondary rhachises 2 to 3.5^{cm} long: peduncles slender, ascending, fascicled by 3's, 4's, and 5's along the terminal portion of the branch, and forming a loose, elongated, relatively narrow inflorescence, leafy towards the base: heads globose, 1.5^{cm} in diameter (incl. the long stamens), roseate; flowers perfect and staminate: calyx campanulate, less than 1^{mm} long, cuspidate-denticulate: corolla 2.7^{mm} long, glabrous, 3–4-nerved and 3–4-toothed; teeth ovate-deltoid, a third as long as the tube: pods 2.5 to 4^{cm} long, glabrous and wholly unarmed both as to valves and replum, long-stiped, torose, acuminate at the tip, 3–4-jointed, Indian-brown at maturity; valves falling away in segments.

Common on hills near Acapulco, Mexico, where collected by Dr. Edward Palmer between October 1894 and March 1895, no. 296.

I am indebted to Dr. Rose for calling my attention to this species, the Palmer's specimen having been undistributed in the Gray Herbarium at the time of my recent revision of the genus. Types in herb. Gray and herb. U. S. National Museum. — B. L. ROBINSON.

THE PROBABLE CAUSES OF THE POISONOUS EFFECTS OF THE DARNEL (*LOLIUM TEMULENTUM* L.).

THE presence of a poisonous principle in the darnel has been well known since the earliest investigations of the subject, and recent experiments confirm this fact. According to Hofmeister,[1] the darnel contains two active principles: *temulin*, obtained by this author as chloroplatinate, which acts upon the nervous system; and the other determined by the oily substances and fatty acids which are contained in the seed in large proportion, which attacks the alimentary canal.

In the course of our researches upon the seed integuments and the pericarp of grasses, we have had occasion to note the practically constant presence in the seeds of the darnel of a fungus to which it seemed reasonable to us to assign the poisonous effects. This fungus, which is always present in the form of mycelial filaments, appears at an early stage in the interior of the ovary. In the first stages of its development it invades the entire nucellus. At the time that the external integument of the ovule disappears, the nucellus itself is almost entirely resorbed, with the exception of two or three of the outermost layers which, obliterated in the maturing of the grain, constitute the hyaline layer. Crowded out by the development of endosperm after fertilization, the mycelium becomes restricted between this hyaline layer and the outermost endosperm. It is in this zone that we have observed it in the mature seed. After the removal of the diverse coatings of the fruit, the hyphæ which constitute this fungus zone appear as septate filaments, generally very long, more or less branched and interwoven with one another. We have found this disposition of the fungus in material from Bolivia, Brazil, Chili, Abyssinia, Persia, Syria, Spain, Portugal, Sweden, Germany, and many localities in France. In [illegible] seeds of most diverse origin the mycelial zone was lacking from the three. This observation has been confirmed in other species of Lolium, to wit, *L. perenne* L., *L. arvense* With. (var. of *L. temulentum*), *L. linicola* Sond. It is only exceptionally that the first of these contains the parasite. The rest are infected to the same degree as *L. temulentum*. When one observes that the species reported poisonous are the very ones in which we have found the parasite, it seems reasonable to ask whether the temulin of Hofmeister is not a result of the action of the fungus upon the nitrogenous materials in the peripheral region of the seed.

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[1] Archiv. f. exp. Path. u. Pharm. 30: —. 1892.

This fungus, of whose nature we are not yet satisfied, may not in any case be identified with the *Endoconidium temulentum* of Prillieux and Delacroix. The latter attacks the seeds of rye which it clearly deforms, the infected grains becoming smaller and lighter than the normal ones. The grains of the darnel show no such deformation. Further, in the rye grains thus attacked, and called "*seigle enivrant*," the protecting layer has generally disappeared, and all the external part of the endosperm has been invaded by the parasite. In the darnel the endosperm suffers no alteration from the action of the fungus, the protecting layer itself remaining perfectly intact. Since our observations were made the same mycelial layer has been noted by Hanausek and Nestler,[2] and before them by Vogl.[3]

Our results are practically alike. However, the other authors have described the fungus for *L. temulentum* alone.—P. GUÉRIN, *Préparateur École Supérieure de Pharmacie de Paris.*

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[2] Berichte der deutschen botanische Gesellschaft 8: 4. *pl.* 12-13. November 30. 1898.

[3] Die wichtigsten vegetablischen Nahrungs- und Genussmittel. 1898.

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OPEN LETTERS.

IN YOUR June number and in a *Bulletin* of the South Carolina Agricultural Experiment Station, Dr. A. P. Anderson quotes me as identifying the Tilletia found on rice in South Carolina with *Tilletia corona* Scrib. The resemblance is certainly striking, but in writing Dr. Anderson I did not intend to express a final opinion in the matter. I had not at that time seen a description of the Japanese *Tilletia horrida* Tak. It now seems to me that the differences in the manner of affecting the host plant, the spore mass being included by the glumes in *T. horrida* and conspicuously exserted in *T. corona* should be considered of sufficient weight to separate the species tentatively until such time as their life histories can be carefully studied. Therefore I should prefer to call the South Carolina specimens *Tilletia horrida* Tak. In my opinion much more confusion is occasioned by the hasty grouping of many forms under one common name than by tentatively recognizing the many forms as independent species.

The name *T. corona* Scrib. seems to be antedated by Arthur's *Ustilago rotundata* (Prel. List Iowa Uredinæ, Nov. 1884), described from Iowa specimens on *Panicum irrigatum*. This species has recently been issued as no. 543 of *Economic Fungi*, under the name *Tilletia rotundata* (Arth.) Ell. & Ev.—F. S. EARLE, *Auburn, Ala.*

CURRENT LITERATURE.

BOOK REVIEWS.

The Sandusky flora.

A LOCAL flora of much interest has been published by the Ohio Academy of Science as the first of a series of "special papers," the second and third numbers of which are devoted to insects. This initial effort reflects credit upon the Academy, both for the subject-matter and for the manner of its presentation. The volume of 167 pages is thoroughly satisfactory in its typographical and press work, and records a piece of careful local exploration worthy of the excellent setting.

The flora of Sandusky and vicinity as here presented is the result of the labors of Mr. E. L. Moseley[1] during the last seven years, and is based upon the herbarium of the Sandusky high school, while specimens of most of the rarer forms are also deposited in the Gray herbarium of Harvard University and in the herbarium of the Ohio University.

The catalogue proper, which is well indexed, is preceded by thirty-four pages of text, in which a number of interesting matters pertaining to the flora are discussed. It adds not a little interest to learn that the flora of Sandusky is exceptional in the number of its species, having more than is given in those excellent floras by David F. Day for the Buffalo region, by Wm. R. Dudley for the Cayuga region, and by the local Academy of Science for the Rochester region, although these regions border on the great lakes and are severally larger than that included for Sandusky. Even the whole of England does not possess a hundred species of phanerogams more than are found about Sandusky. The islands included in the area, a half dozen having been explored and studied with special care, do not add materially to the richness of the flora, as they have only a few plants not found on the mainland. The surprising abundance of species is ascribed to favorable physiographical conditions in part, but more especially to the climate. The summer is longer at Sandusky than at other points along the shore of Lake Erie, being much over a month longer than at Buffalo, and the spring and summer have a higher average temperature. The reasons for this state of things are discussed and statistical evidence presented.

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[1] MOSELEY, E. L.: Sandusky flora, a catalogue of the flowering plants and ferns growing without cultivation in Erie county, Ohio, and the peninsula and islands of Ottawa county. Special paper No. 1. Ohio Academy of Science; Wooster, 1899. 8vo. pp. 167, 1 map.

The nomenclature of the catalogue is that of the *Kew Index*, with concession to the last edition of Gray's *Manual*. The enumeration be with *Botrychium ternatum* Swartz and ends with *Xanthium Canadense* Altogether it is an excellent catalogue, and reflects credit upon the in and ability of the author, and upon the enterprise of the Academy of Sc —J. C. A.

Anatomy of the dicotyledons.

A SENTENCE of Radlkofer, which the author thinks prophetic— next hundred years belong to the anatomical method"—inspired Solere to bring together the immense mass of material regarding the anatomy stem and leaves of the dicotyledons into a handbook for botanical tories.[2] To the previously accumulated knowledge of anatomy the himself has been a notable contributor.

The labor of compilation alone must have been immense. Here brought together in systematic form the data regarding each of the fam of dicotyledons. The author first presents a synoposis of the chief anat cal characters of the family as a whole; then gives an extended accou the leaf structure, followed by a similar presentation regarding the st Each section closes with a thorough bibliography.

In an introductory chapter Dr. Solereder explains what is meant by anatomical method, and discusses the more important anatomical charac and their value in taxonomy. An extensive closing chapter (75 pages) devoted to a synopsis of the various anatomical features, with reference their occurrence in certain families, genera, and species—a sort of compa tive anatomy.

Though one may doubt whether the anatomical method is destined to play the important rôle in taxonomy which Radlkofer and his pupil Solere believe, and may easily find matter for criticism in the introduction, as w as flaws in the details regarding structure, the enormous labor which author has performed in the production of the work disarms criticism a evokes only praise. The book is unquestionably a most useful one for ref ence, not only to the systematist, but to the histologist and to the physio as well. It will prove indispensable in every botanical department whe active work is in progress and will doubtless demonstrate its value from to day.

As a reference book it has one serious defect, the want of a full index. The index only includes the families, and while ordinarily this will give desired clue, the value of the book would be greatly enhanced by an entr each genus and species and of each author mentioned. The Bavarian Academy of Sciences, which has assisted in its publication, might well have increased its subvention if necessary to provide such an index.—C. R. B.

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[2] SOLEREDER, HANS: Systematische Anatomie der Dicotyledonen. Ein Ha buch für Laboratorien der wissenschaftlichen und angewandten Botanik. 8vo. xii + 984. *figs. 189*. Stuttgart: Ferdinand Enke. 1898–1899. *M* 12.

Speculative biology.

In 1875 Pflüger propounded a hypothesis regarding the constitution of organized bodies which may be described as the hypothesis of chemical continuity. Impressed with the extensive polymerization among carbon compounds, especially the proteids, he ventured the suggestion that in an organism polymerization may progress indefinitely, so that the whole protoplasm is not an aggregate of similar molecules having definite molecular weight but may form a single giant chemical molecule. This theory has found few adherents. It is accepted *in toto*, however, by Dr. Georg Hörmann, who proceeds in a recent book[3] to show its adequacy to explain certain biological problems, and, therefore, its inherent probability.

He applies it to the transmission of the impulse in nerve and the phenomena of nerve section; to the contraction of muscle and the discharge from the electric organs of fishes; and discusses the structure of the cell and the rotation of the protoplasm "from the standpoint of the *principle* of chemical continuity." (Hypothesis — principle: are they synonymous?)

Of course the book is pure speculation, and must not be taken as anything else, though we fear the author does not always remember the sandy foundation on which he is building. The various ingenious diagrams, representing atoms of divers interesting forms and positions lend an air of verisimilitude which might deceive the very elect.

Theory we recognize as necessary; speculation is indispensable in the formation of multiple working hypotheses by the investigator; but it may be seriously doubted whether the publication of a speculation is ever worth while. Until we have more intimate knowledge of the chemistry of proteids, speculation of the kind here set forth must be regarded as little more than vanity and vexation of spirit.—C. R. B.

NOTES FOR STUDENTS.

CONTINUING his observations on the agencies by which insects are attracted to flowers, Professor J. Plateau now gives a large number made on *Salvia horminum* and *Hydrangea opuloides*,[4] confirming his previous statement that they are chiefly attracted by the sense of sight. Neither the colored bracts in the former nor the conspicuous sterile flowers in the la plant can be regarded as "vexillary." In both cases the pollinating inse make their way at once to the flowers which contain the honey without be visibly guided by the showy organs in either case; while if these are rem it does not appear to make any material difference in the number of inse which visit the inflorescence.— Jour. Roy. Mic. Soc. 1899. 298.

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[3] HÖRMANN, GEORG: Die Kontinuität der Atomverkettung ein Strukturprinzip der lebenden Substanz. 8vo. pp. iv + 118. *figs. 32.* Jena: Gustav Fischer. 1899.

[4] Mém. Soc. Zool. de France 11: 339–375. *fig.* 4. 1898.

IN A NOTABLE paper on cellulose enzymes, Professor F. C. Newc clearly demonstrates the existence of cytohydrolytic enzymes distinct fr diastase, especially in the seedlings of white lupine and date palm.[5] T enzymes, which in some plants are doubtless mixed with diastase, "a starch so feebly and on reserve cellulose so energetically that they are regarded as cytase as distinguished from diastase." In all cases the walls first become hyaline, then more and more transparent, finally see to melt away in solution. Besides the clear proof of the existence of the suspected cytase, the paper adds much to our knowledge of the distribu of cellulose enzymes.— C. R. B.

DR. A. M. BOUBIER in a brief paper on the pyrenoid[6] states that his observations "prove the existence in pyrenoids of an external plasmic membrane perfectly differentiated and independent of the chromatophore, at least at its mature stage of development. This membrane encloses a leucoplast, wh accumulates starch, with a crystalloid at the center. -- C. R. B.

DR. G. HOCHREUTINER has determined experimentally that seeds number of aquatic plants may pass uninjured through the alimentary can of herbivorous fish, and the latter may therefore aid in the dissemina aquatic plants.[7]— C. R. B.

THE CHIEF VALUE of Kolkwitz's recent paper on the influence of light on the respiration of fungi[8] is due to the refinement of technique employed the degree of accuracy attained. In these respects it surpasses pre work and eliminates many sources of error that have been neglected. is the first extensive accurate study of the effect of light upon the res tory activities of plant protoplasm and of animal protoplasm as well. mals are prone to move and then by their varying activities to render any conclusions as to the effect of light alone. Severed parts of plants unsuitable, as diffusion at the cut end is abnormal and quantitatively alte Fungi having false parenchyma are unsuitable since the intercellular s may suffer change from light. Therefore the author selects such fungi as produce a loosely woven mycelium that spreads itself out openly to the light (Aspergillus, Penicillium, bacteria), and measures such activities as are solely dependent on light. As sources of error he recognized chiefly the evolution of CO_2 through decomposition of oxalic acid or of dead parts, and errors introduced by variation in temperature. The classical method of Pettenkofer (1862) is adopted and the amount of evolved CO_2 is determined by titration with oxalic acid. To secure greater accuracy the gas was forced, not drawn, through the apparatus at the rate of three, four, or five liters per hour as desired. The process and apparatus are described in great detail. Suffice it to say here that exceeding care was given to every feature. The culture vessel, of special design, presented a great surface to the light while of but small capacity. In order to reduce the absorption by glass the walls of the vessel were very thin. The feature wherein this study chiefly surpasses previous work is in the regulation of the temperature of the culture during experimentation. This was accomplished by immersing the culture vessel in a tank containing six liters of water and keeping it at a constant temperature by electricity, automatically regulated by a very ingenious contrivance. Lest the thin layer of water covering the culture flask should vary in temperature all of the water was continually agitated by a paddle operated by a turbine. The inflowing air was warmed to the temperature of the water. In this way the variation was from one tenth to one thirtieth of a degree C. The electric light was constant in quantity and quality, thus avoiding the variations inevitable in the use of sunlight. Estimations of CO_2 were made every two minutes. The author announces as a result of his labors that light, under the conditions employed, increases respiration about 10 per cent. The effect is observable in young or old cultures, richly or poorly nourished fungi, and in acid or alkaline media. The influence of light during long periods when secondary processes arise was not investigated. An excellent bibliography is given.—F. L. STEVENS.

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[5] Annals of Botany **13**: 49–81. 1899.

[6] Bulletin de l'Herbier Bossier **7**: 451–458. 1896.

[7] Bulletin de l'Herbier Boissier **7**: —. 1899.

[8] KOLKWITZ, R: Ueber den Einfluss des Lichtes auf die Athmung der nie Pilze: Jahr. f. wiss. bot. **33**: 129–165.

ITEMS OF TAXONOMIC interest are as follows: In continuing his flora of the West Indies (*Symbolæ Antillanæ*), URBAN, in the second part, presents the Araliaceæ, represented by four genera and sixteen species; and in another paper describes about eighty miscellaneous new species, chiefly from Puerto Rico, among which we notice two new genera, *Notodon* (Leguminosæ, near Sabinea) and *Hybosperma* (Rhamnaceæ). LINDAU presents the Polygonaceæ, represented by ten genera and sixty-six species, the great genus Coccoloba containing about fifty of them. SCHLECHTER presents the Asclepiadaceæ, represented by twenty-one genera and eighty-eight species, the largest genus being Metastelma, with thirty-four species, eighteen of which are new. Two new genera are established, *Tainionema* and *Decastelma*.—K. MIYAKE (*Bot. Mag. Tokyo* 13:1–4. *pl. 3.* 1899) has described a new genus of Hepaticæ,

very closely resembling Pellia. It is said to have a spermatozo
larger than that of Pellia, which has heretofore been credited with the
spermatozoids among the Hepaticæ. The new genus is known as *M*
in honor of Makino the discover. Specimens without sporogonia had
been described by Stephani as *Pellia crispata*, so that the name sta
M. crispata (St.) Miyaki.—In *Proc. Amer Acad.* (**34**: 507–534. 18
INSON and GREENMAN publish revisions of Montanoa, Perymenium,
uzania, long a puzzling series of Mexican and tropical American com
Montanoa is recognized as containing thirty-two species, nine of which
Perymenium has twenty-six species, ten of which are new; and *Z*
has twelve species, two of which are new. The same authors (*ibid.* 53
have published a synopsis of the genus *Verbesina*, which has not been
as a whole since 1836 (DC. Prodr.), at which time thirty-three specie
recognized, all but two being American. Now the genus is conceded
exclusively American, and contains 109 species, more than 70 per ce
which are local. The greatest display of species is in the uplands of
and southern Mexico, where 40 per cent. of the species are endemic.
synopsis twenty-five new species are described.—GREENMAN (*ibid.* 5
has published some new and critical Mexican species, the new species
bering twenty.—ELIAS NELSON has published a revision of the ph
western North America. He recognizes thirty-eight species, nineteen
are new. The paper is a master's thesis in the University of Wyom
is published in the ninth report of the Wyoming Agricultural College.
mie, Wyoming.—E. P. BICKNELL, in continuing his studies of Sisyr
(*Bull. Torr. Bot. Club* **26**: 297–300. 1899), has described four new s
from Michigan.—A. A. HELLER (*ibid.* 312–315) has described add
new species from western North America.—AVEN NELSON (Erythea 7
1899) has discussed the western species of *Aragallus* (*Oxytropis*), des
eight new species; and has also described (*ibid.* 65–70) five new for
Oreocarya, two of Cryptanthe, and one of Allocarya.—JARED G. SMITH
18. Div. Agrost. U. S. Depart. Agric.) has published a synopsis of the
Sitanion, recognizing twenty-three species, twenty of which are new.—

GUIGNARD has recently studied the reduction of chromatin in
major[9]. This plant has proved exceptionally favorable for such a study,
the number of chromosomes, twelve in the sporophyte and six in the
phyte, is the smallest yet reported for any flowering plant.

First division.—In the prophase the spirem splits longitudinally a
segments into six primary chromosomes each of which consists of two
During the succeeding contraction and growth, each of these pieces sh

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[9] Le développement du pollen et la réduction dans le *Naias major*. Arch.
microscopique **2**: 455–509. 1899.

double row of chromatin granules, a preparation for a second splitting, so that the primary chromosomes are to be regarded as quadruple. As the primary chromosome separates into its two parts (secondary chromosomes), the splitting already inaugurated by the fission of the granules begins to take place, but is not entirely completed, since the two chromosomes remain united at their extremities, thus forming a **V** with its apex attached to the contractile threads of the spindle. Each daughter nucleus receives six double (secondary) chromosomes.

Second division.—In the second division, six **V**-shaped chromosomes appear. At the point of the **V** there is an interruption in the linin support and everything favors the conclusion that these are the secondary chromosomes of the first division which have not lost their individuality. No longitudinal division takes place at this time, there being merely a distribution of the two parts of the **V**-shaped double chromosome. Thus the two divisions merely distribute the four parts of the primary quadruple chromosome (tetrad), which were already defined in the prophase of the first division. It is evident that there can be no qualitative reduction.

Miss Sargant[10] both figured and described a second fission of the chromatin granules in *Lilium Martagon*, and called attention to the quadruple nature of the primary chromosome. Guignard believes that his results agree with Farmer's account of Pallavicinia, Brauer's of Ascaris, Meves' of Salamandra, and also with Belajeff's description of Iris, although that writer has given a different explanation of the origin of the chromosomes in the pollen mother cell. An excellent review of the chromosome problem, illustrated by very clear diagrams, is by no means the least important part of the work.

The brilliant results of Nawaschin and Guignard on the fertilization of Lilium have been confirmed by Miss Sargant,[11] who finds that during fertilization the male nucleus is applied to the female nucleus, while the second male nucleus is applied to both the polar nuclei. In one case, in which the polar nuclei were not yet in contact, the much elongated "antherozoid" united them like a bridge. In several preparations it was noted that the pollen tube, after fertilization had taken place, contained two small nuclei. Since both generative nuclei are already accounted for, it is suggested that these are probably due to the division of the tube nucleus.—CHAS. J. CHAMBERLAIN.

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[10] Ann. Bot. 11: 187–224. 1897.

[11] On the presence of two vermiform nuclei in the fertilized embryo-sac of *Lilium Martagon*. Proc. Royal Soc. 65: 163–165. 1899.

NEWS.

A BIOGRAPHY and bibliography of the late Professor William Nylan appears in the June number of the *Revue général de Botanique* (11: 218-2

IN AN APPENDIX to the *Bulletin* of miscellaneous information issued the botanical department of Trinidad, the superintendent, Mr. J. H. Har is giving a description of the West Indian and Trinidad ferns.

MESSRS. CARLTON R. BALL, of Iowa Agriculture College, Elmer D. M rell, of the University of Maine, and P. Beveridge Kennedy, of Cornell Un sity, have been appointed assistants in the Division of Agrostology, U Department of Agriculture.

DR. FRANCIS RAMALEY, of the University of Minnesota, has bee appointed professor of biology in the University of Colorado, at Boulder succession to Professor John Gardiner, who has retired on account of continued ill-health, having held the chair since 1889.

THE Académie Internationale de Géographie Botanique has confer its international scientific medal upon Professor John M. Coulter. T medal is awarded by the Academy "in recognition of services rendere science by those who cultivate and advance its various branches."

A WORK by Dr. C. B. Davenport, of Harvard University, intended e cially for botanists and other naturalists who are interested in the qua tive study of species and of organic variation, is announced by John W & Sons, New York City. It is entitled "Statistical methods with spe reference to biological variation."

PROFESSOR DR. EDUARD JANCZEWSKI, Cracow, Austria, is engaged study of the geographical distribution and the forms of *Ribes rubrum*. desires to examine the forms growing in the United States, and partic the var. *subglandulosum*. Anyone who can furnish a range of herba specimens and viable seeds of these forms will confer a favor by comm cating with Professor Janczewski.

A "MANUAL of the flowering plants of Iowa," by T. J. Fitzpatrick Lamoni, Ia., is announced. The publication of this work is a private e prise, and intending purchasers should address the author. Part I. c ing the Polypetalæ, is not a mere list, but each species is briefly des together with notes on habitat and distribution, with suitable analytic ke

A serious disease of the peach appeared in Michigan and other states several years ago and seems to be spreading. It is known to growers as "little peach" for the reason that the fruit of the affected trees seldom attains more than one fourth normal size. The Division of Vegetable Physiology and Pathology of Washington is making a study of the disease this year, the work being placed in charge of Mr. Merton B. Waite, who is now in the field.

Mr. Hermann von Schrenk has been made a special agent in the Division of Vegetable Physiology and Pathology of Washington and authorized by the Secretary of Agriculture to make such studies of the diseases of forest trees and timber as may be directed by the chief of the division to which he has been appointed. This important line of work has been much neglected in this country, and it is hoped that with the start made it will soon have the attention it deserves.

Mr. W. A. Orton, who has for several years been associated with Professor L. R. Jones of the University of Vermont, has been made an assistant in the Division of Vegetable Physiology and Pathology, U. S. Department of Agriculture. Mr. Orton will spend the greater part of the summer in South Carolina and other southern states, investigating the diseases of cotton. He is now at James island, S. C., making some preliminary studies of a disease of sea island cotton which has prevailed in that section for several years.

Mrs. Arvilla Bacon Ellis, wife of Mr. J. B. Ellis, of Newfield, N. J., died at her home on July 18, after a long illness, at the age of sixty-eight. While Mrs. Ellis was not known as a botanist, it was her cooperation which made possible the issue by her husband of the two great sets of fungi exsiccati, the *North American Fungi* and *Fungi Columbiani*. The 3000 portfolios in which the sets were mounted were made by her own hands; and she arranged and folded the pockets for about three fourths of the 200,000 specimens sent out in those books. Her labor of love certainly deserves public recognition.

An international conference on hybridization was held in Chiswick Garden, London, July 11 and 12, under the auspices of the Royal Horticultural Society. An exhibition of hybrids was given on the first day in the large vinery in the Gardens. The conference was attended by many distinguished botanists and horticulturists. Mr. H. J. Webber was the delegate from this country. With the aid of lantern illustrations he described the hybridization work which he and Mr. Swingle are prosecuting under the charge of the Department of Agriculture. Professor L. H. Bailey sent a paper, but was unable to be present in person.

The Yale Corporation, at a recent meeting, voted that the chair of botany held by the late Professor Daniel C. Eaton until his death should hereafter be known as the Eaton Professorship of Botany. It may be recalled that this professorship was founded and endowed by a relative and by friends of Professor Eaton in the year 1864, although it has never been distinguished by a name. The extensive and valuable botanical library and herbarium which Eaton accumulated have been donated to the university by his family, and Mrs. Eaton has placed in the botanical laboratories a bronze tablet to her husband's memory, and, in addition, has founded a graduate scholarship in botany.—*Erythea*, for August.

PROFESSOR BESSEY has been called upon to serve as the Acting Chancellor of the University of Nebraska, and as the duties of the chancellorship will make it necessary for him to be relieved of much of his botanical work in the laboratories and lecture room, the regents have elected Dr. August Rimbach (Ph.D., Jena 1887) *ad interim* Instructor in vegetable physiology and pathology. Dr. Rimbach is known as the author of many botanical papers in the *Berichte der deutschen botanischen Gesellschaft* and other German botanical journals. He was a pupil of Stahl, Detmer, Schwendener and Sachs, and was Professor of Botany in the University of Cuenca, Ecuador, from 1889 to 1894. He has traveled extensively in South America engaged in the study of flora of tropical and alpine regions.

MR. J. N. ROSE has just returned from a three months' trip through Mexico, bringing about 900 species of dried plants, many living plants, especially agaves, and plant photographs. His collection is not so large nor so rich in new species as the one of 1897, but it will doubtless help to clear up many puzzles which have long worried botanists dealing with Mexican plants. Besides rediscovering *Echinocactus Parryi*, he collected several other species lost or hitherto unknown to American herbaria. About 200 species were collected at type localities. Among the places visited were Chihuahua, Aguas Calientes, Jalapa, City of Mexico, Oaxaca, Cuernavaca, Pachuca, Real del Monte, Guadalajara, Tequila, San Luis Potosi, and Guanajuato; all places from which many types have been taken. Mr. Rose also made a very thorough study of the species of agave, especially those used in the manufacture of pulque and mescal. The visit to Tequila was chiefly to learn what plant furnishes the "tequila" which is the great mescal drink of Mexico.

Mr. Rose was accompanied by Dr. Walter Hough of the National Museum.

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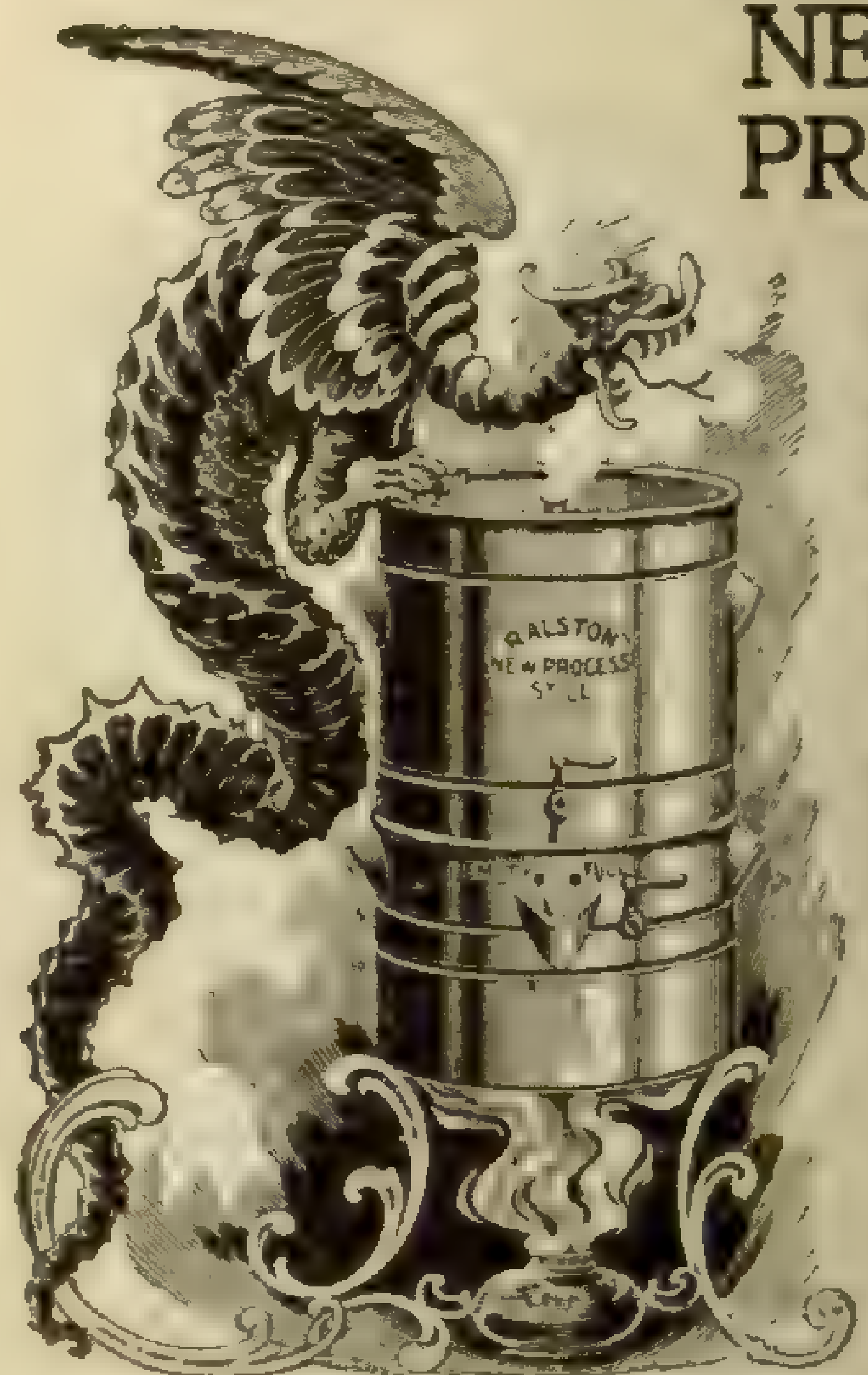

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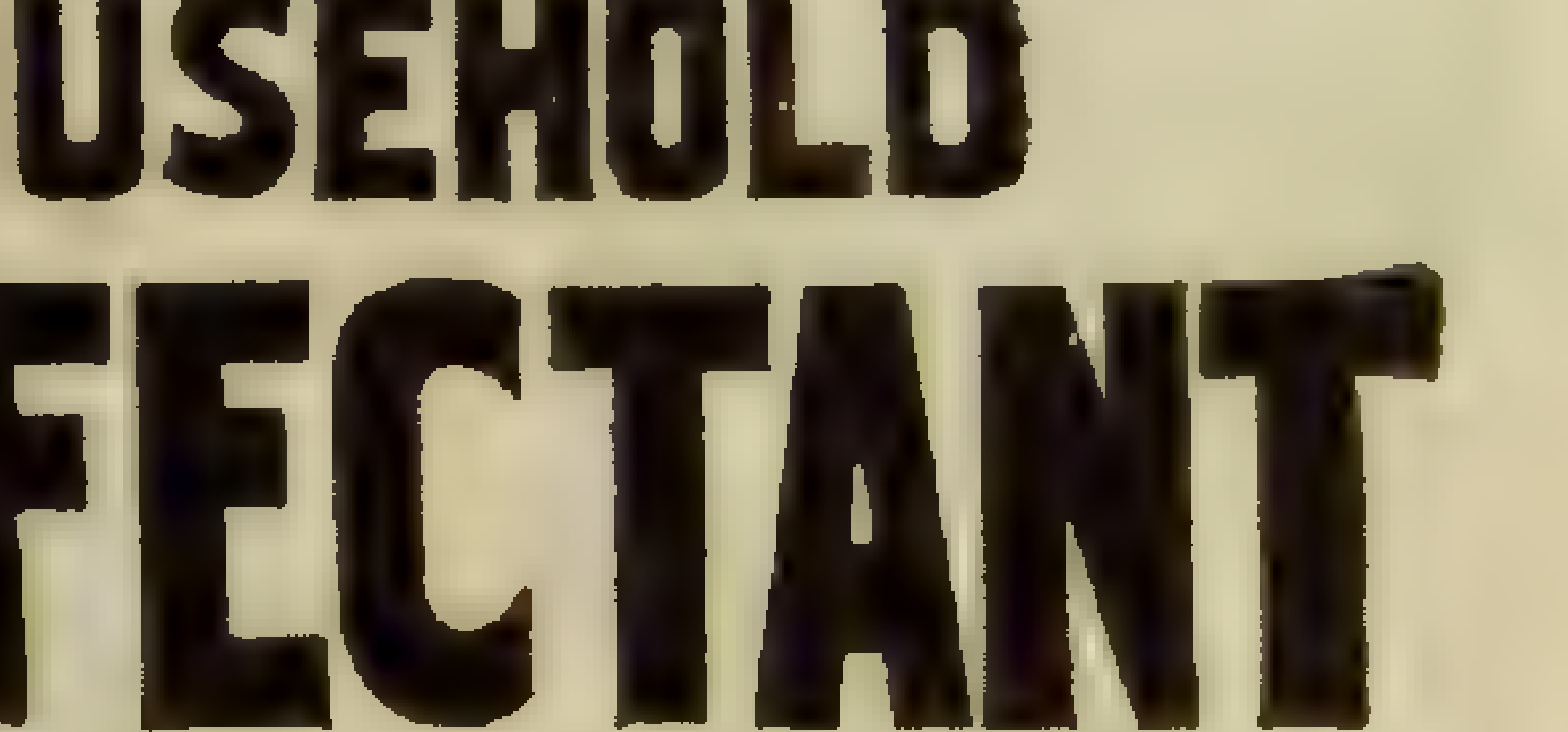

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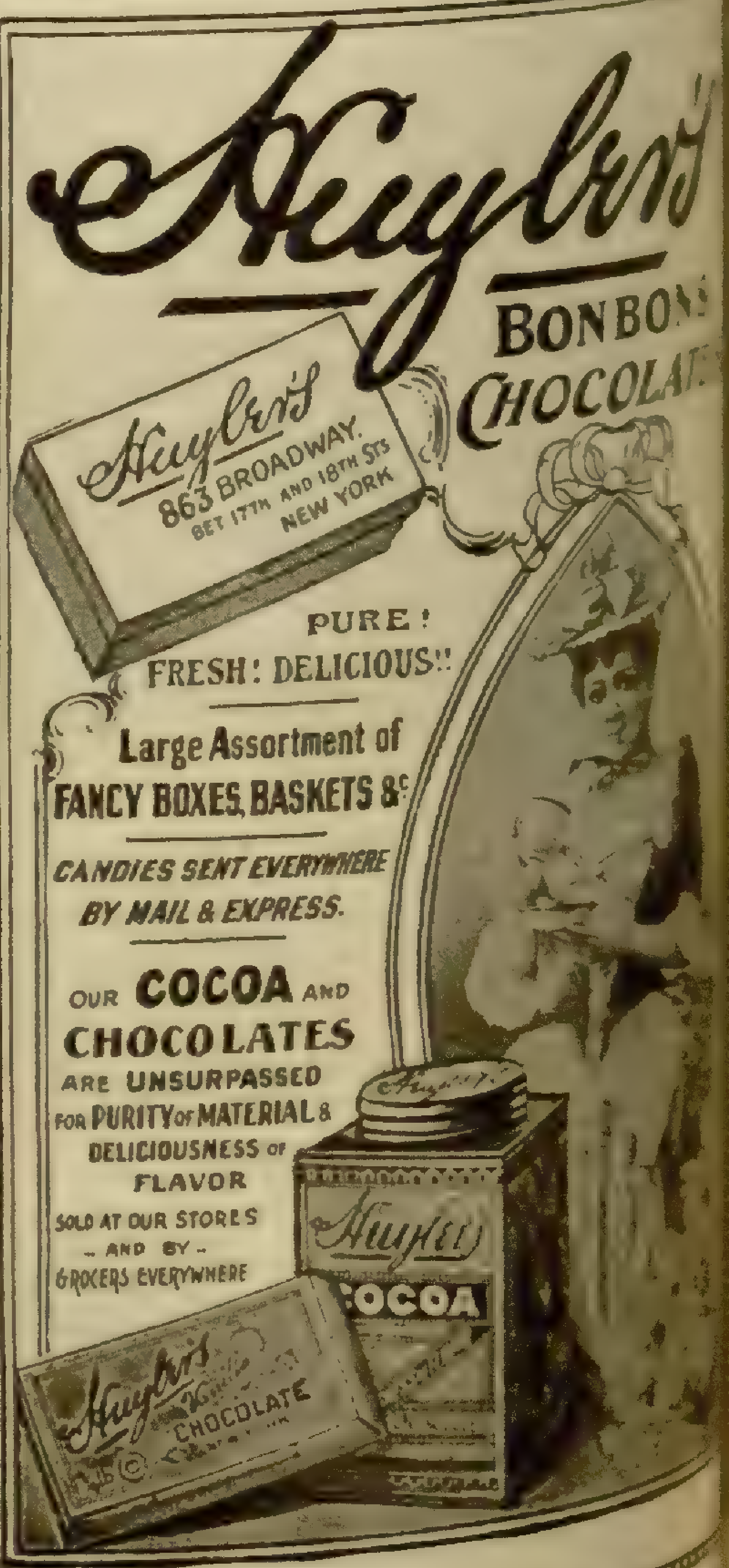

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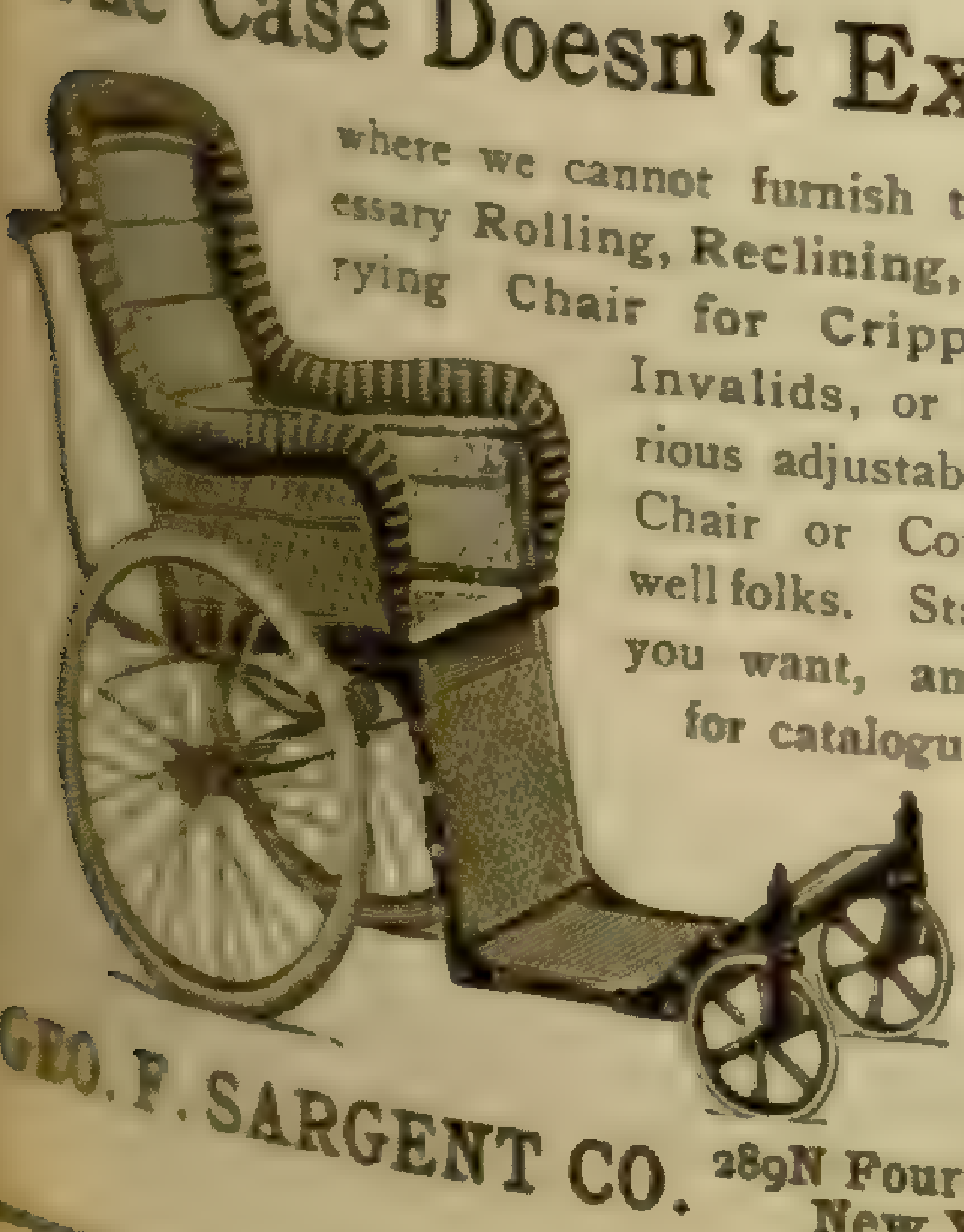

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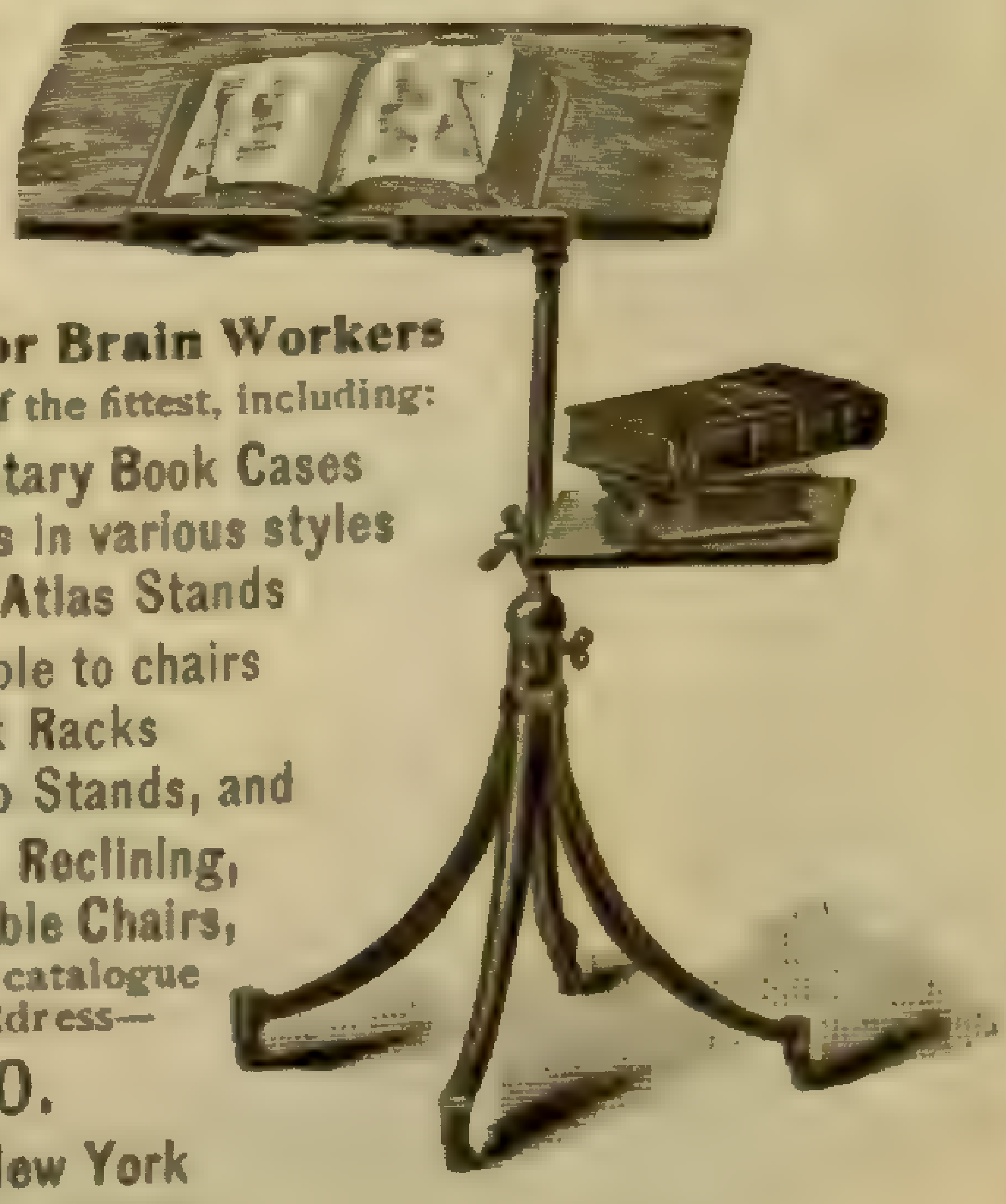

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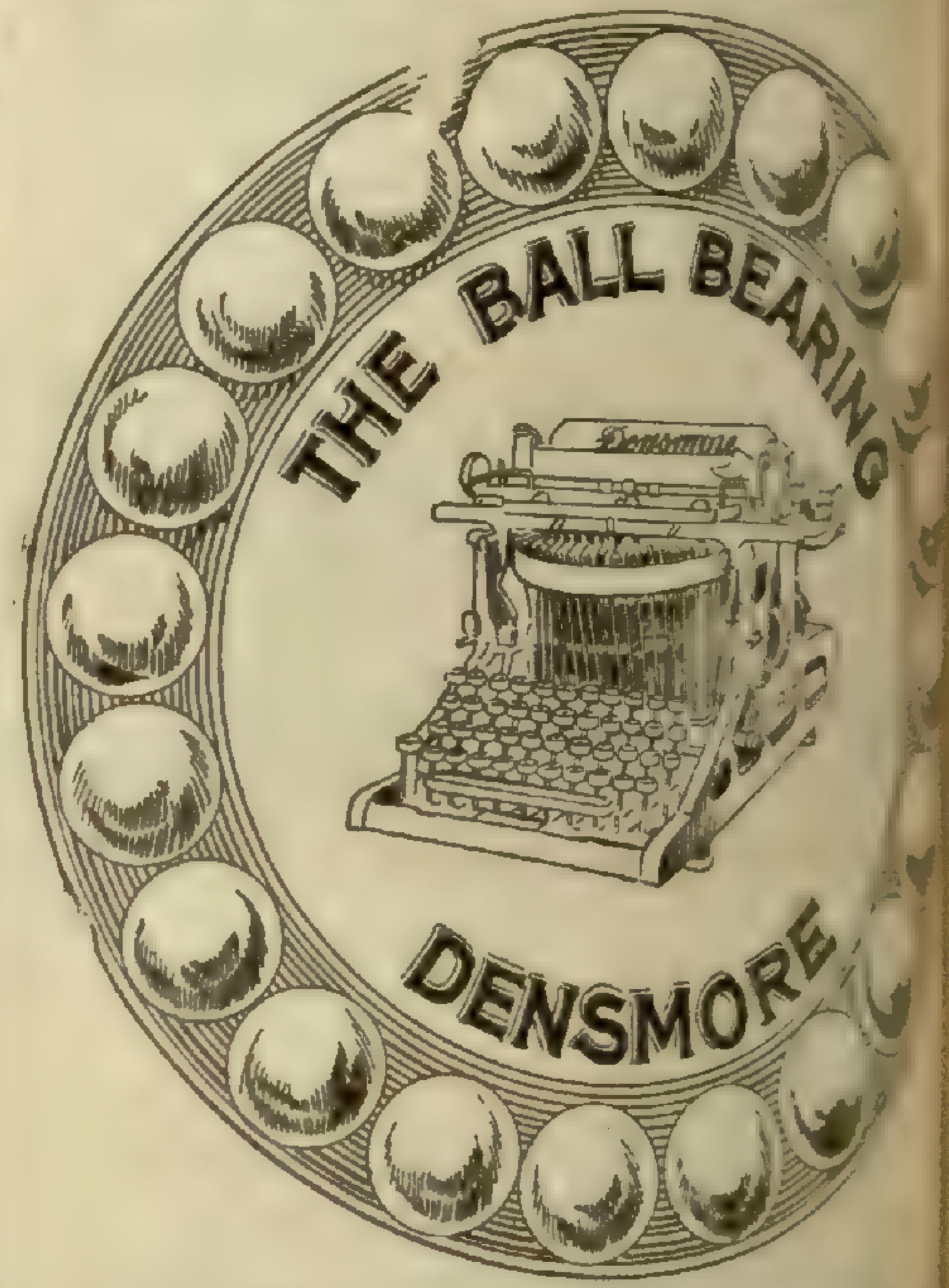

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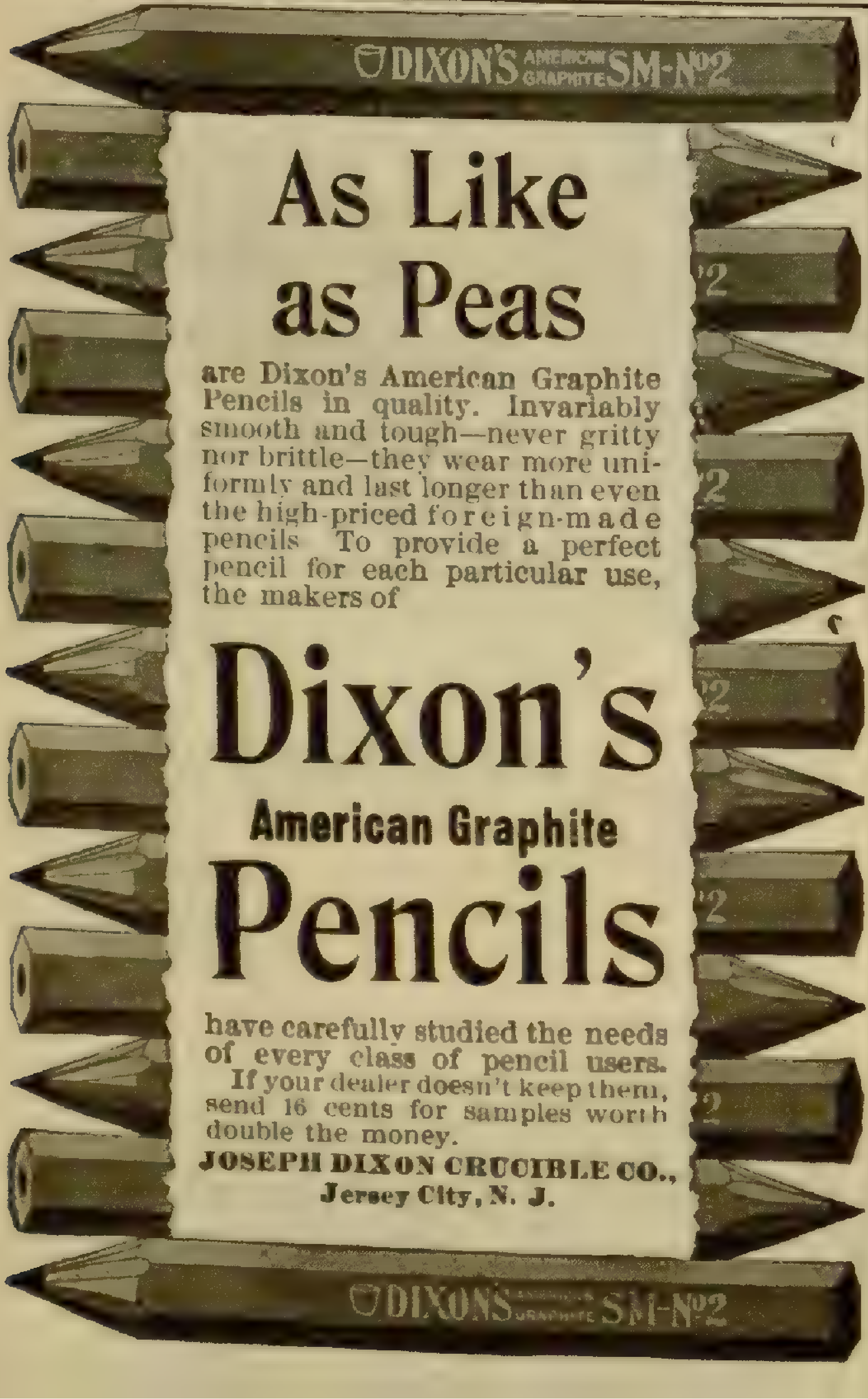
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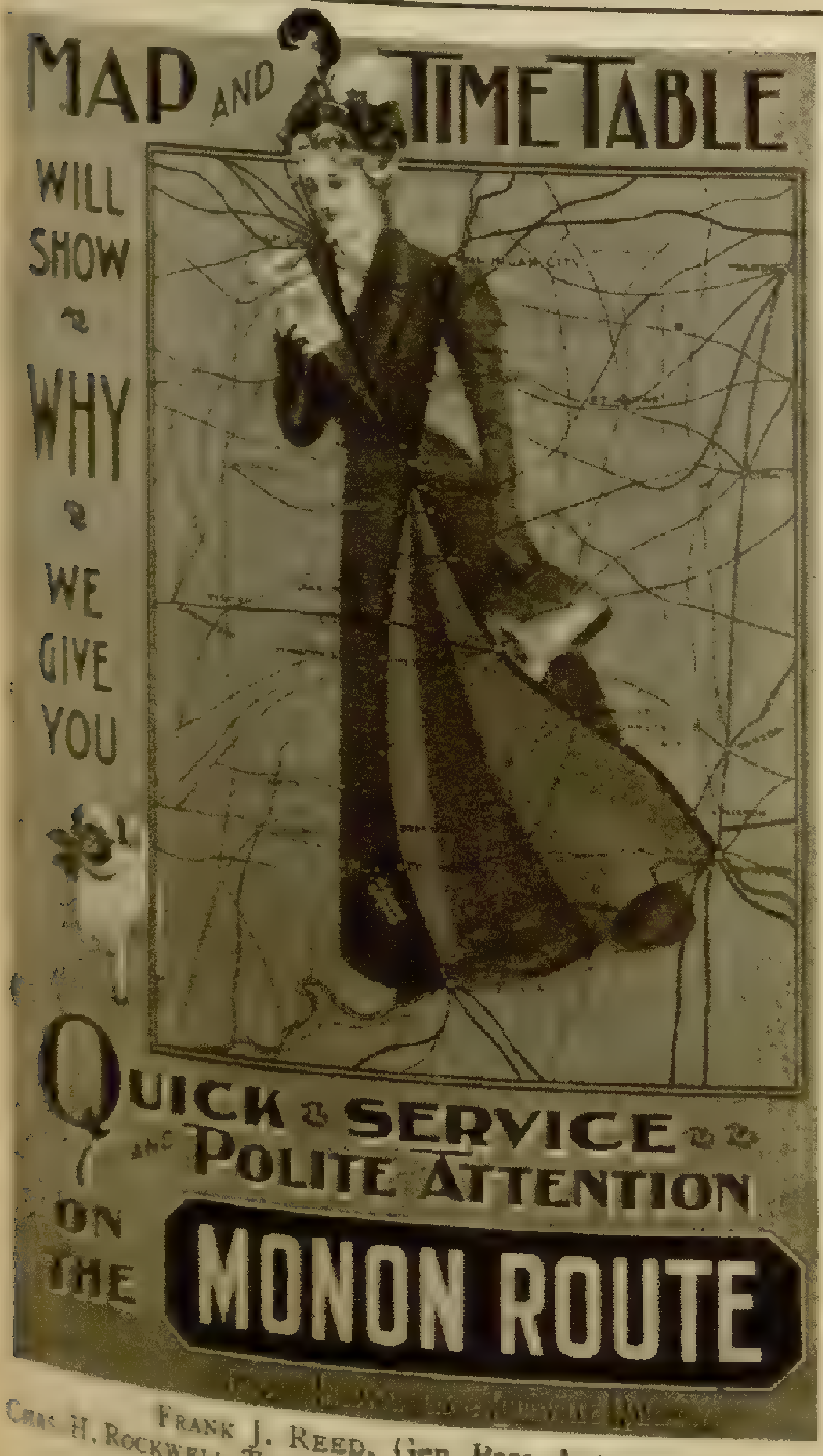
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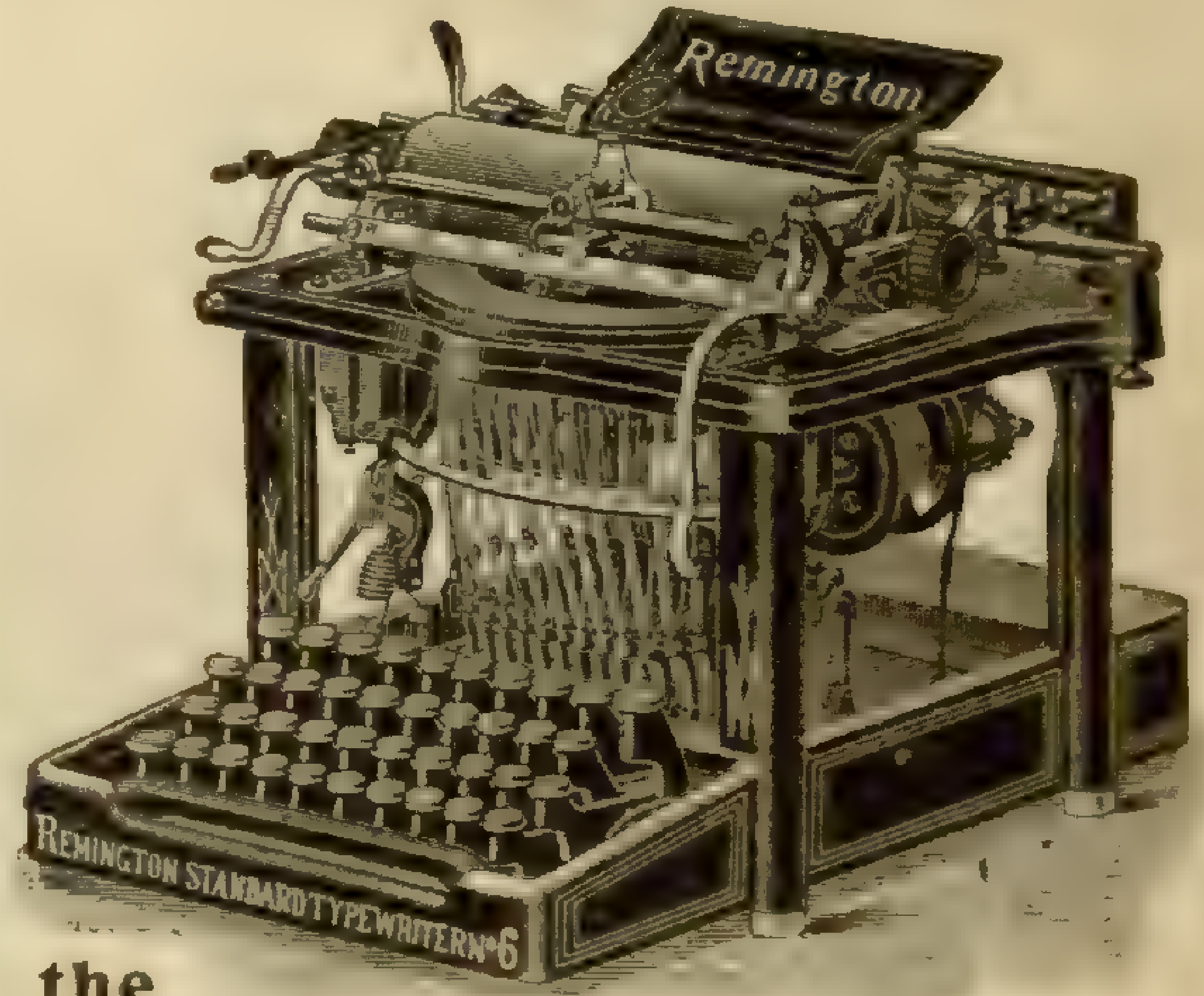
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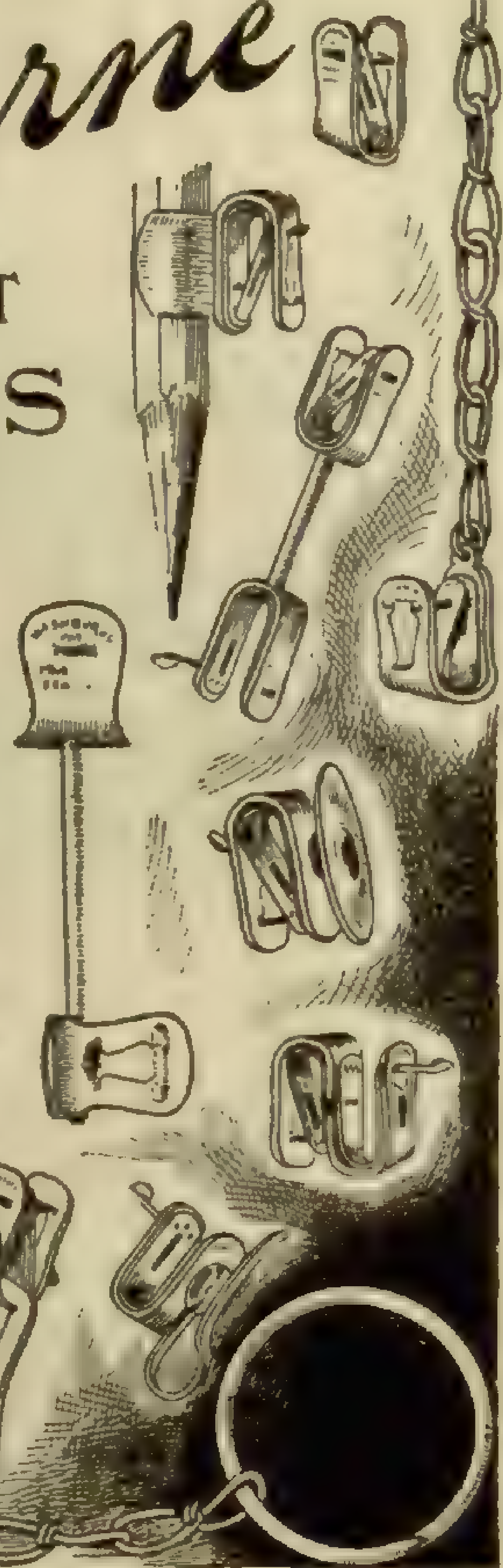

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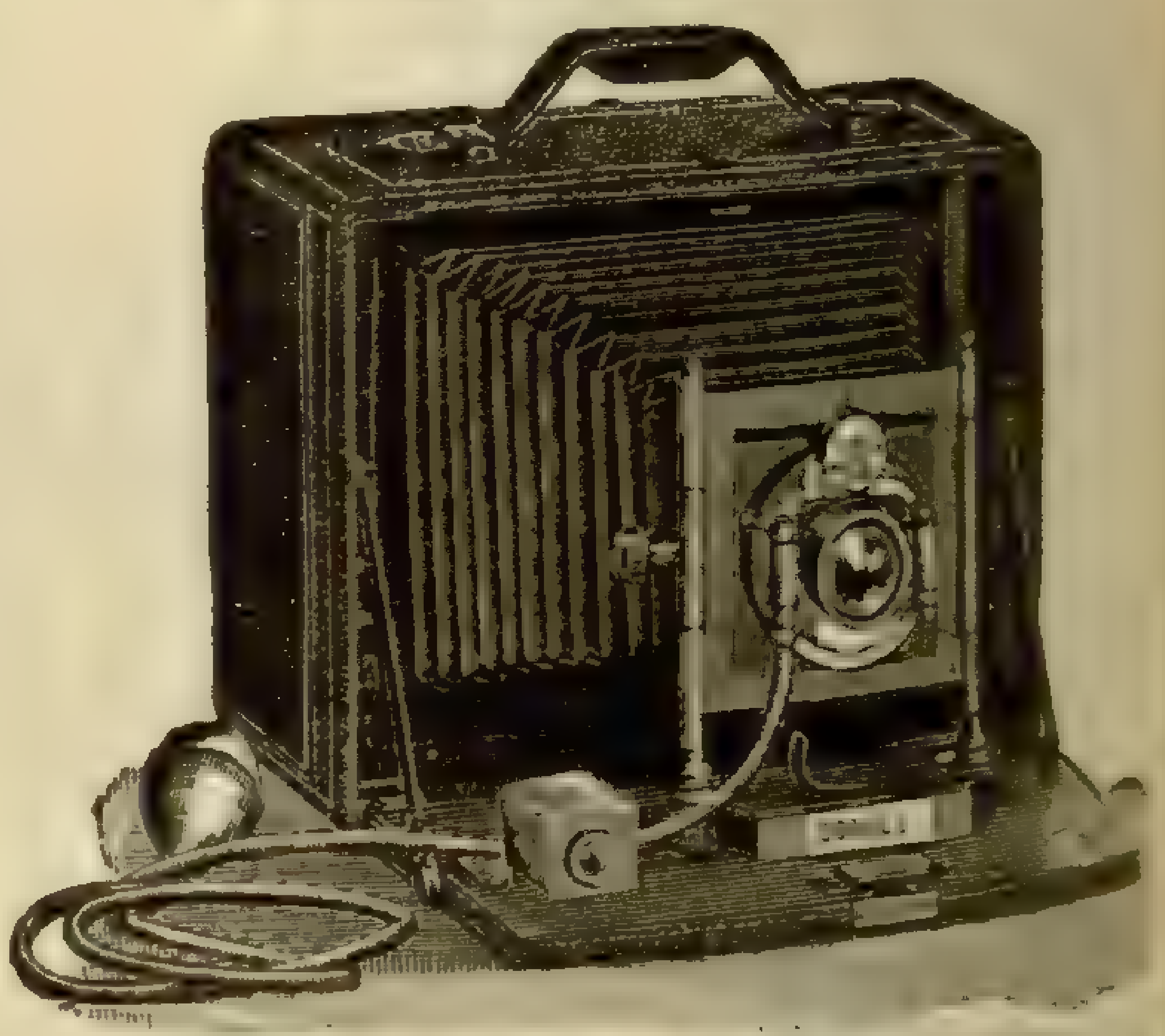

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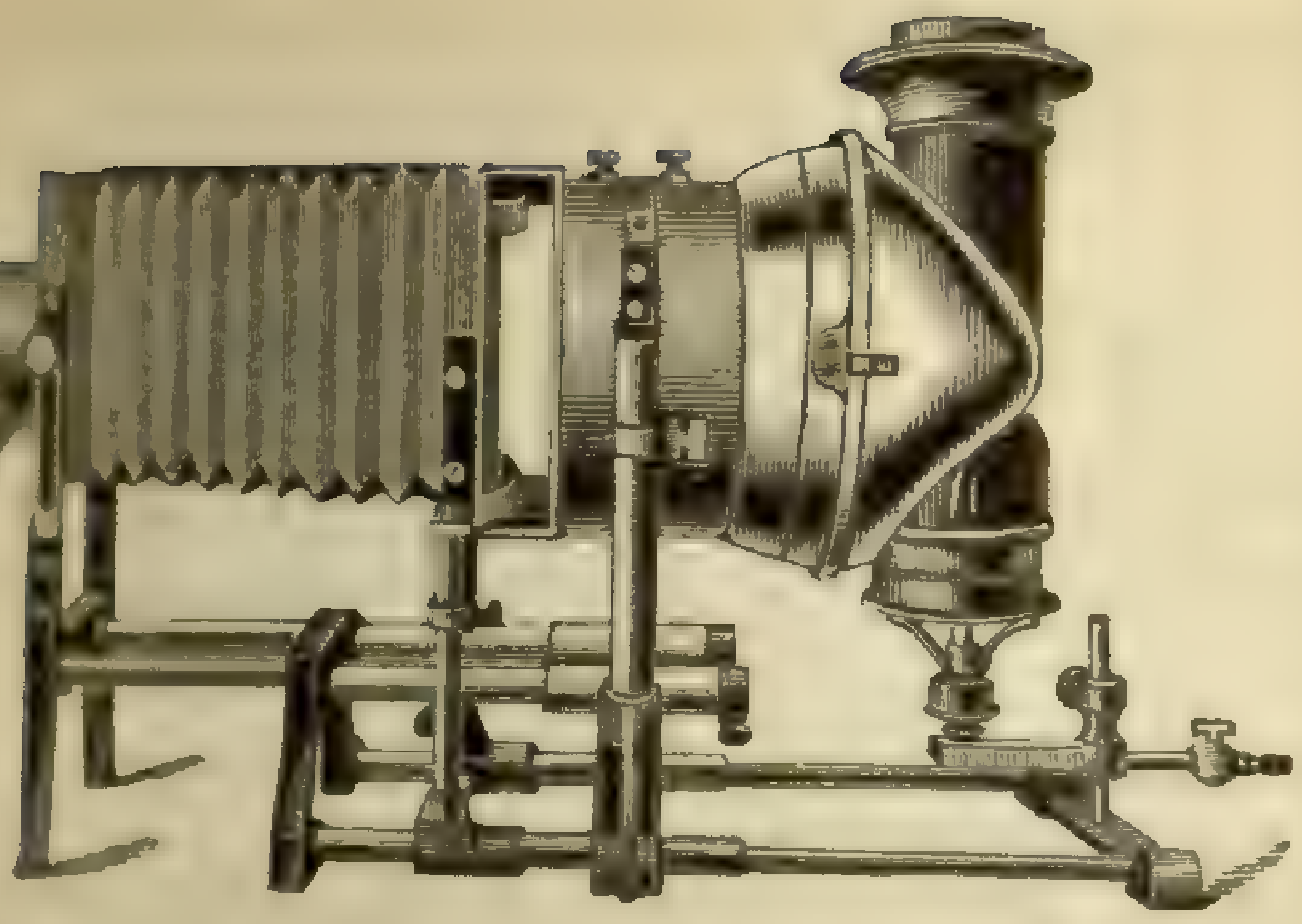

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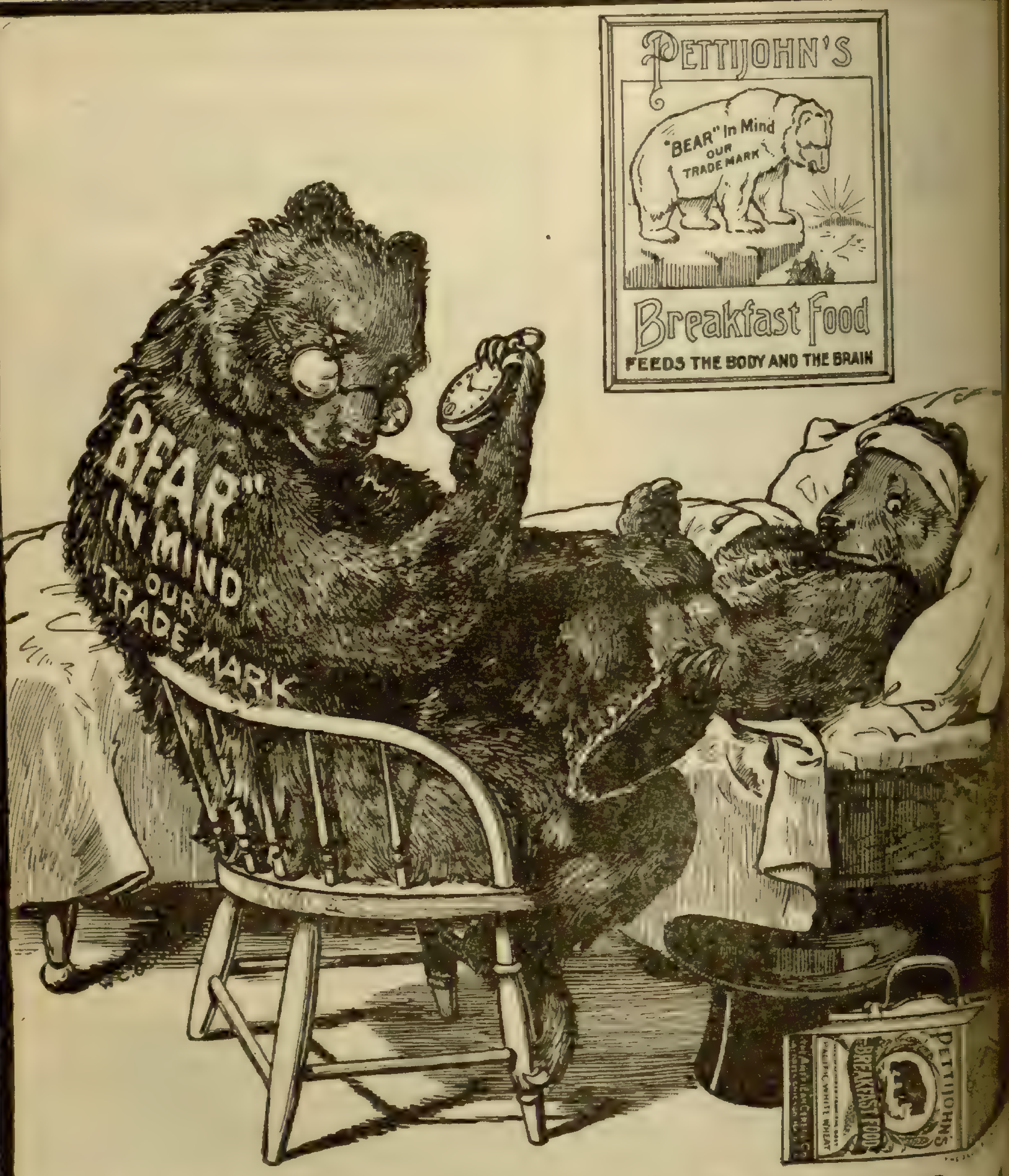
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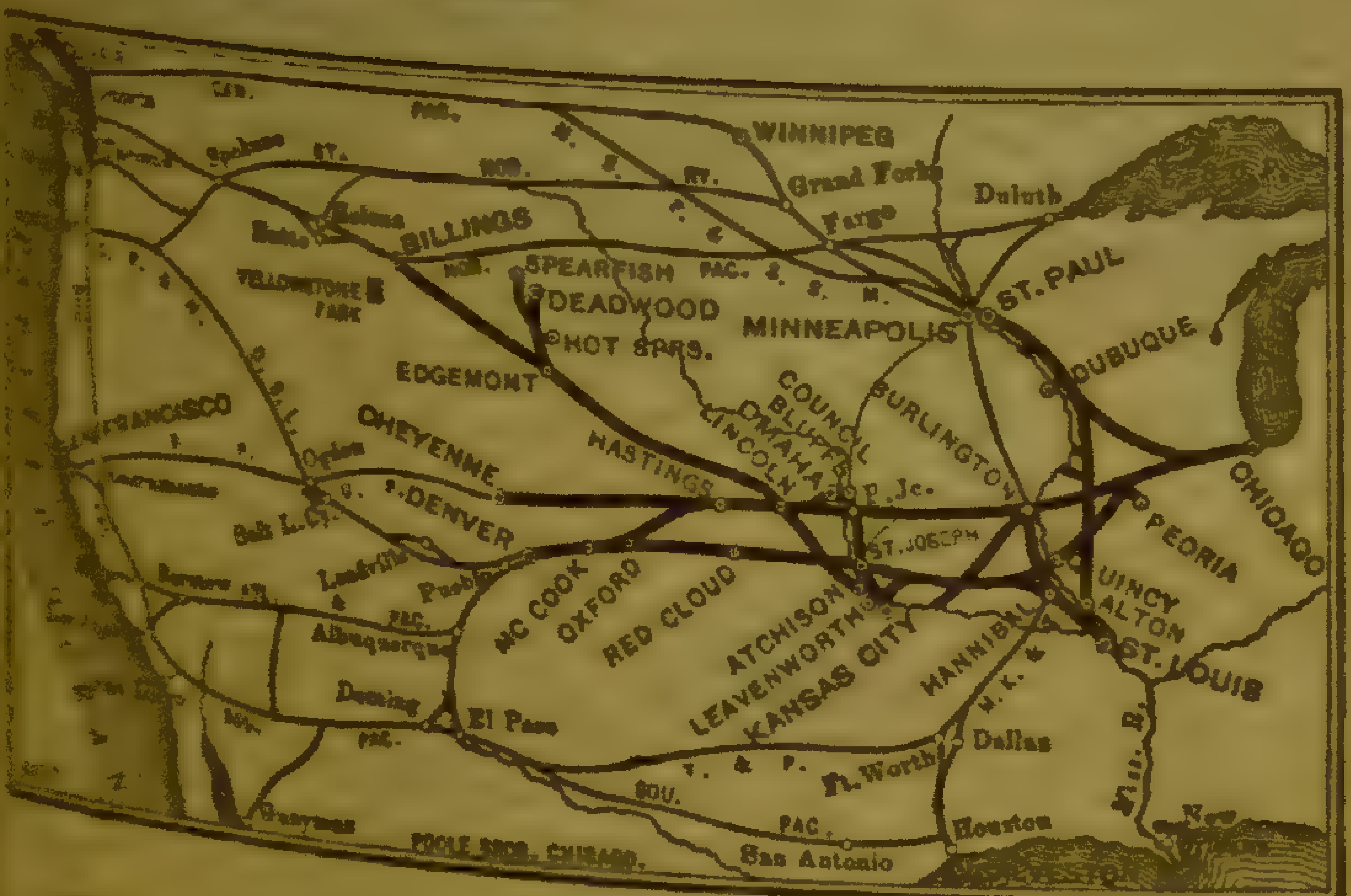
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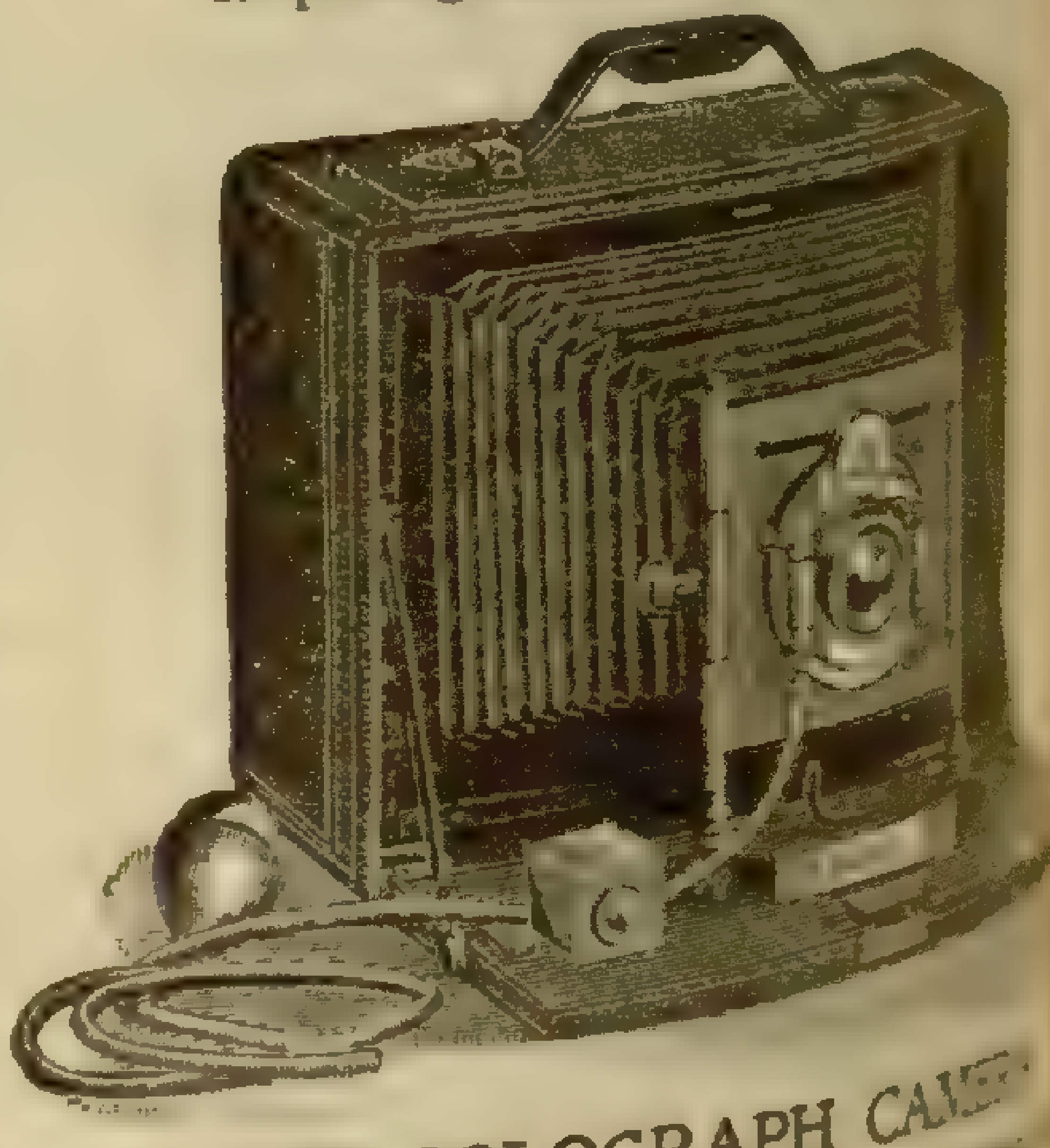

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VOLUME XXVIII NUMBER 3

BOTANICAL GAZETTE

SEPTEMBER 1899

THE COMPOUND OOSPHERE OF ALBUGO BLITI.

CONTRIBUTION FROM THE HULL BOTANICAL LABORATORY. XVI.

F. L. STEVENS.

(WITH PLATES XI–XV)

THE results of this investigation introduce factors entirely new in their essential features and at variance with the cytological processes connected with fertilization previously described for coenocytic or other forms of plants or animals. The title of the paper must suggest a contradiction of terms. The writer hesitated before using the expression compound oosphere, but it seemed better, at least for the present, to modify the term oosphere which has become firmly fixed in descriptive botany so that it would express the peculiar conditions here set forth. If future investigations should indicate that the peculiarities of the compound oosphere are not exceptional, it may be necessary to introduce a new term indicating the conditions and the process of fertilization presented in this paper.

A compound oosphere is one containing several or many functional sexual nuclei. This idea violates the present conception of the structure of that cell as it exists throughout the plant kingdom, and furthermore, so far as the writer is able to judge, the ova of animals present no such peculiarity. If there is any character that defines the oosphere and ovum it appears to be the presence in the cytoplasm of a single female nucleus that is normally fertilized by a single male nucleus. In *Albugo Bliti* Biv., however, the mature oosphere contains many female nuclei, and fertilization is effected by the discharge of many male nuclei from the antheridial tube and their subsequent fusion with the female nuclei in pairs. An oospore results from this multiple sexual act with about one hundred fusion nuclei, which remain in the resting condition until germination. The existence of such conditions must be supported by strong evidence, and great caution should be exercised in interpreting the data upon which the conclusions are based.

One is partially prepared, however, for the acceptance of such conditions as these by the thought that the form under consideration is a coenocyte, and that comparatively little is known of the behavior of the nuclei and cytoplasm in such structures. Excellent summaries of current knowledge are given by Humphrey ('92), Zimmerman ('96), and Wager ('96). It is unnecessary, in view of the existence of these accounts, to enter into details here. Suffice it to say that in several (Monoblepharidinaceæ, Entomophthoraceæ, and Chytridineæ) of the main coenocytic groups the behavior of the nuclei in fertilization is practically unknown. In those groups of which there is more knowledge (Saprolegniaceæ, Peronosporeæ, Zygomycetes, and Siphoneæ) concordant results have not yet been attained. For example, in the Saprolegniaceæ the question is still in dispute whether or not fertilization occurs (Hartog '95, Trow '95). In the Siphoneæ the two most comprehensive papers (Behrens '90, Oltmanns '95) upon the one genus (Vaucheria) that has been investigated disagree essentially as to the events leading to the development of the oosphere. The process of fertilization described for the Zygomycetes by Léger ('95) involves the unique phenomenon of the fusion of nuclear complexes.

The pioneer work on the histology of the Peronosporeæ, done by Wager on *Peronospora parasitica* in 1890, was followed by article by the same author in 1896 on *Albugo candidus*. Berlese in 1898 published an article on the entire group. It appears that most of the study of the histology of coenocytic fungi has been concerned with the Peronosporeæ, but even here the data are yet too scanty to admit of any wide generalizations. The improvements in technique during recent years should be held in mind, as much of the discrepancy between the earlier and later results may be thus explained.

Wager's work (**'96**) should be consulted for a comprehensive summary of the knowledge up to the time when he published the results of his investigations. For *Albugo candidus* his own research shows a condition where the antheridial tube liberates one sperm nucleus which fuses with the solitary female nucleus in the ooplasm. He employed corrosive sublimate in saturated aqueous solution as a fixing agent, and stained the sections with Hartog's nigrosin-carmine. In his earlier work on Peronospora Wager describes a multinucleate oogonium and antheridium. The nuclei of the maturing oogonium pass to the periphery, where they divide mitotically. Two or three then return to the center and probably fuse, as only one nucleus is found there at a later stage. The antheridial nuclei divide simultaneously with those of the oogonium. The antheridial tube contains one or more nuclei, the antheridium finally having many less than it had at an earlier stage. This material was fixed by either absolute alcohol or chromic acid, and stained with Kleinenberg's hæmatoxylin.

Berlese gives four figures to illustrate the development and fertilization of *A. Portulacæ*, but his description of the nuclear transformation is not illustrated. All statements concerning Albugo seem to have been based on either this species or herbarium specimens of other species. He studied also four species of Peronospora. For a killing agent he used either 95 per cent. alcohol, alcoholic corrosive sublimate, Flemming's solution, or picric acid, and stained with Flemming's triple stain or Hartog's nigrosin-carmine. Further reference will be made to these articles later in the paper.

The results presented in this paper were obtained mainly from material fixed in chrom-acetic acid, cut in serial sections in paraffine, and stained on the slide by Flemming's triple stain. For full details regarding methods the reader is referred to the end of the paper. This investigation was begun in 1897, one year being spent in the botanical laboratory of the Ohio State University through the kindness of Dr. W. A. Kellerman. I am indebted to Mr. J. H. Schaffner for many courtesies during my work at the same institution. Since the summer of 1898 the study has been continued under the direction of Dr. Bradley Moore Davis in the Hull Botanical laboratory of the University of Chicago, where I have also received helpful advice and suggestions from Dr. J. M. Coulter and the members of the botanical staff. I wish to express my thanks to my wife for much kind assistance, in particular for the preparation of Plate XV.

DEVELOPMENT OF THE OOGONIUM AND ANTHERIDIUM.

The character of the mycelium of Albugo varies with the nature of the host tissue. The hyphae are slender where the cells of the host are thick-walled and placed close together, while in loose tissue they may swell to a considerable diameter. *Fig. 42* shows the general structure of the hyphae, the rounded nuclei, each with a prominent nucleolus and membrane, being distributed irregularly through the vacuolate cytoplasm. A single nucleus is represented in *fig. 1*. It is worthy of attention principally because of its very faint linin network. It has an actual diameter of from 2–2.5 μ.

The oogonium may be terminal or intercalary, its walls being simply the greatly expanded mycelial wall, as is evidenced by the frequent persistence of haustoria on its surface. Even very early stages of developing oogonia may be distinguished from enlarged mycelia by certain peculiarities of the protoplasm (*fig. 43*.) The nuclei are elongated, the vacuoles are angular and distorted, and the cytoplasm is drawn into stringy bands; which gives evidence of a disturbance not present in the ordinary mycelium. These peculiarities are frequently evident in vegetative hyphae a distance of 200 μ from the developing oogonium.

similar appearances were noted by Istvanffi ('95) and Wager ('96), and their explanation is undoubtedly the true one, namely, that the protoplasm was rapidly flowing from the mycelium to fill the enlarging oogonium. When sufficient nucleated protoplasm has entered the developing oogonium, this structure is cut off from the hyphae by a septum at the point of enlargement (*fig. 44*). The oogonium is now fully differentiated from the vegetative hyphae, the nuclei recover their original form and lie in a coarsely vacuolate and somewhat granular cytoplasm. The general appearance of the oogonium and its contents may be seen in *fig. 45*.

The antheridium develops simultaneously with the oogonium, but gives no evidence of the flowing of the protoplasm into the growing structure. Probably owing to the small size of this organ there is but little disturbance as it fills with protoplasm. It becomes cut off from the parent hypha and the contents are similar in appearance to those of the oogonium as is shown in *fig. 45*.

The most conspicuous feature in this early development of the oogonium is the increase in the size of the nuclei. This seems to occur somewhat rapidly just before the oogonium has attained its full size. As the nuclei grow larger the linin network becomes much more prominent, until finally it assumes a very characteristic structure in the form of large meshes, the threads being coarse, densely staining, and apparently homogeneous in structure. The whole appears to be a connected network that lies against the nuclear membrane. When the threads are seen in transverse view they appear as round or oblong bodies about the size of the nucleoli, but staining differently. This condition of the nucleus, which is shown in *fig. 3*, may be regarded as the spirem stage of early mitosis. While the nuclei are passing into this condition the oogonium wall thickens slightly.

It is at this time that the number of nuclei may be determined most easily and accurately. The count ranged from 226 to 333, the smaller number being quite exceptional. Making allowance for the fact that several nuclei may readily have been counted twice in adjacent sections, it would perhaps be fair to place the average at about 250. Wager found 115 in *A. candidus* and Berlese 200 in *A. Portulacæ*. It is very difficult to make an accurate count in the antheridium, because this structure is so small and of such irregular shape that it is impossible to recognize its limits in adjacent sections. However, an average based on several counts indicates that the number is likely to be about 35. This number is considerably greater than that suggested by either Wager or Berlese, both of whom report about 12 nuclei in the antheridium.

When the oogonial nuclei are passing into the spirem condition the *Hautschicht* seems to be closely appressed to the wall in the vicinity of the antheridium. This fact is demonstrated most clearly in preparations where there has been slight collapse of the contents of the oogonium (*figs. 47, 48*) and the protoplasm has shrunken away from the wall everywhere excepting at the point opposite the antheridium. This adherence of the *Hautschicht* is correlated with a very marked granulation of the cytoplasm in this region, a phenomenon also noted by Wager, and one which seems to be significant. It suggests that a cellulose enzyme is secreted to dissolve the wall of the oogonium. As indicated in *figs. 48, 49, 50*, this wall frequently shows the marks of corrosion over a considerable area, always at a point opposite the antheridium. This interesting process results in a neat perforation, through which the cytoplasm of the oogonium flows so as to form a very conspicuous swollen papilla within the antheridium. Various stages in this process are shown in *figs. 50–*. It is difficult to explain this phenomenon. The initiatory step in the perforation of the oogonium wall seems without doubt to be taken by the protoplasm of the oogonium itself. But what is the significance of the pushing of the cytoplasm of the oogonium into the antheridium to form the peculiar bubble-like papilla? The structure, both wall and contents, stains deeply, thus becoming very conspicuous, while its extremely frequent occurrence, as well as its presence in other species, seems to indicate that it is not abnormal. The papilla wall is so extremely thin that may only conjecture whether or not it is derived from the antheridium wall. The history of the papilla beyond the stage shown in *fig. 54* is not clear. In this condition the structure is very thin-walled and its contents vacuolate, resembling a large irregular compound bubble. Whether the delicate wall now bursts or the contents are gradually withdrawn into the oogonium is uncertain. At all events, the papilla in later stages leaves no trace of its former existence. There follows at a later period conditions (*figs. 56, 57*) which show that there is certainly a movement of the cytoplasm in the opposite direction, the antheridium extending a process with a cell wall through the opening into the oogonium. The first surge of the cytoplasm from the oogonium into the antheridium may be due simply to the unequal conditions of turgor in the two structures, but it is possible that there is also a phylogenetic significance in the phenomenon. The occurrence of similar structures in *A. candidus* and *A. Portulacæ* shows it to be of some import. Such a papilla in a much less highly developed form is figured by Wager and referred to as the receptive papilla.

The antheridial tube presses into the oogonium in the form of a slender thin-walled process (*figs. 56, 57*). It is filled with dense cytoplasm that greedily absorbs and retains stain, and is surrounded by a sheath of dense oogonial cytoplasm. The nuclei remain in the antheridium, none entering the tube at this time, and are indistinguishable in size and structure from those in the oogonium. They are also in the spirem condition, similar to that described for the oogonial nuclei. The description of the further development of the antheridial tube is deferred, to follow the account of the differentiation of the compound oosphere.

DIFFERENTIATION OF THE COMPOUND OOSPHERE.

The previous description carries the history of the sex organs up to a time when the antheridial tube has penetrated the oogonium one fourth or one fifth the diameter of that structure. Correlated with the further development of the tube there occurs the differentiation of the periplasm and ooplasm, and the extrusion of the nuclei from the central region of the oogonium. The process consists essentially in a centripetal movement of the cytoplasm, and results in a massing of this cytoplasm in the center of the oogonium in such a manner that the vacuoles and nuclei are carried to the periphery of the denser central portion thus developed. Behrens ('90, 315) describes a somewhat similar condition in Vaucheria as follows: "Der ganze Vorgang besteht also in der Ablösung des grössten Theils der Protoplasten von der Wand durch Vacuolisation der wandständigen Plasmaschichten." This curious phenomenon was noted by Wager in *A. candidus* and subsequently by Berlese in *A. Portulacæ.* The process as heretofore described is simple. In *A. Bliti* it is complex, but unique and full of interest; and as a complete knowledge is essential to an understanding of the further development of the oosphere a detailed description must be given.

The first hint of the centripetal aggregation is found in a tendency of the cytoplasm to depart from the even distribution shown by a young oogonium, and to collect in masses throughout the interior (*fig. 58*). These denser portions run together forming fewer but larger masses (*fig. 59*). Thus several prominent aggregations of cytoplasm may be formed, separated from one another and from the wall by vacuoles of varying sizes (*fig. 60*). These denser regions are homogeneous in structure, containing minute vacuoles of uniform size evenly distributed in a matrix of cytoplasm free from granules. The dense regions contain no nuclei, because these are forced from the dense cytoplasm to a position on its periphery. The dense centers now coalesce forcing out the vacuoles. This may result immediately in the condition shown in *fig. 61*, but frequently the coalescence proceeds more slowly and irregularly, and often a reniform mass is formed, the indentation on one side marking the juncture yet to be made. The last gap narrows until only a few vacuoles remain to mark its track (*fig. 61*), and these soon float outward leaving one mass of cytoplasm, the rudimentary oosphere (*figs. 62, 64, 65*). As the vacuoles pass outward they often leave captive nuclei in their wake (*figs. 61, 62, 64*), but these soon follow. A typical v of the resulting condition is presented in *fig. 64*. The outer region of the oogonium, the rudimentary periplasm, is coarsely vacuolate, presenting a conspicuous contrast to the dense central mass. Along the boundary between these two regions are gathered most of the nuclei (*fig. 64*).

The next stage in the differentiation of the oosphere is conspicuous and clearly characterized. It ends in producing a distinct differentiation between the oosphere and the periplasm (*fig. 65*). This condition is brought about primarily by the marshaling of the nuclei into an oval or an irregular hollow sphere, a section of which is shown in *fig. 65*, while a somewhat earlier stage is to be seen in *fig. 64*. Both figures illustrate the one important fact that all or nearly all of the nuclei are at the boundary of the central dense mass. The latter figure in addition shows that there is a sharp line of demarcation between the ooplasm and periplasm. There are usually a few scattered nuclei in the periplasm, and occasionally one finds a nucleus in the oosphere that has not passed out as rapidly as the others.

Important changes occur in the cytoplasm while the nuclei are arranging themselves into a hollow sphere. At the beginning of this process the region that is to become periplasm is coarsely vacuolate, in marked contrast to the dense cytoplasm of the center, but the two regions blend gradually together where they meet (*figs. 62, 64*). Later when the hollow sphere of nuclei becomes more regular in outline dense granular cytoplasm is differentiated around and between the nuclei (*figs. 65, 68, 69*). The inner border of the rudimentary periplasm also becomes differentiated into a film more densely granulated than any other region of the oogonium, and finally determines the limit of the oosphere. There is not yet an organized wall, and the most critical study reveals nothing more than a dense film of protoplasm. It is convenient to call this condition the stage of zonation.

The position of the stage of zonation in the sequence of events leading to the differentiation of the oosphere is clear. With this condition comes the characteristic and sharp limitation between ooplasm and periplasm which is maintained until maturity, while before zonation such a differentiation did not exist. The process of differentiation is gradual, and a series of developmental stages has been obtained which seems complete. This period is the only one where the ooplasm possesses very few nuclei or none, and it is impossible to regard it as being a period later than stages which present zonation and also contain 50–100 nuclei (*figs. 68, 69, 70*). The development of the antheridial tube is such as to lend strongest support to the sequence above indicated, since the tube is shorter in stages preceding zonation and longer in stages following it (see plates), thus affording strong corroborative evidence. While the differentiating line is characteristic of zonation the paucity of nuclei in the ooplasm is equally so. The sharper the differentiation the fewer the nuclei, and when zonation was very definite none could be found, and it is probable that when this stage is at its highest development there are no nuclei in the ooplasm. There is some evidence, however, that makes it seem possible that one and even two spindles sometimes remain in the ooplasm, but this is uncertain.

No mention has been made, as yet, of the division of the nuclei of the oogonium. This mitosis closely accompanies the process of zonation as is indicated in most of the figures. These two events apparently take place nearly simultaneously. The earlier prophase is typical in such stages as are shown in *figs. 58, 59, 60, 61*. At the time of complete zonation the nuclei are in metaphase and lie close to the line that separates the ooplasm from the periplasm (*figs. 65, 66*). Spindles are frequently found that actually cross this line at right angles, so that one pole is in the ooplasm and the other in the periplasm (*figs. 65, 66*). The mitoses that take place at this period mark an important and characteristic phase in the history of the oogonium. The dividing nuclei that lie tangential to or wholly outside of the boundary line between the ooplasm and periplasm leave their daughter nuclei in the periplasm. Each of the spindles which cross the line (*fig. 66*) gives one daughter nucleus to the oosphere and the other to the periplasm, and the line of differentiation is sharply defined and unmistakable. Nuclei may be observed in every phase of this mitosis, and the daughter nuclei may be found in all stages of reorganization, one of each pair in the ooplasm the other in the periplasm. The writer was unable to detect any difference between the mitoses that occur strictly in the periplasm and those that contribute daughter nuclei to the oosphere. As a result of the division a large number of nuclei pass into the ooplasm, thus producing a multinucleate cell containing by actual count an average of 45 to 55 nuclei (not less than 40 or more than 60). The oosphere is thus a coenocyte; instead of the uninucleate cell which one would expect there is found a multinucleate structure, to designate which the writer has used the term compound oosphere.

Because of the importance of the anomalous compound oosphere and the peculiarities of its development, it seems best to discuss at length difficulties that might be suggested. It may be claimed that a mistake has been made in the sequence of events, and that the multinucleate condition of the central region does not follow, but precedes zonation. This objection is invalid, for four reasons: (1) the sequence is complete up to zonation, and there is no place for a multinucleate central region in the series; (2) the studies in cytology make it certain that there is but one simultaneous mitosis of the oogonial nuclei, so that the phase of the division serves as an index of the age of the oogonium, thus rendering any misunderstanding of the sequence impossible; (3) the interpretation presented is necessary to the understanding of stages that are positively and unmistakably older; (4) the development of the antheridial tube and central body correlate with the views presented. It would seem impossible that a mistake in seriation has been made. It may be claimed that the daughter nuclei seen in the mature oosphere are not *in situ*, but were carried in by the knife. There is abundant evidence to controvert this claim, for the nuclei in anaphase often lie in such positions that, if carried in by the knife, they must have been carried in two opposite directions by the same stroke. It may be well to state that specimens of every structure or stage represented or described in this article are preserved, not in one, but in several mounts, and for most of the important stages in many preparations. No isolated or single fact is anywhere used either to support or destroy any theory.

With the entrance of the nuclei differentiation of the oosphere is complete. The ooplasm is of very fine, even texture, made up of such small meshes that the vacuoles are never more than half the size of the nuclei, and there are no prominent granules or oil drops shown by the Flemming stains at this time (*figs. 61-69*). The periplasm is loosely vacuolate, the strands are often granular, and the nuclei are frequently at their intersections, often in bunches.

The nuclei of the antheridium usually divide simultaneously with those of the oogonium, this being so constantly true that from a glimpse of one organ the condition of the other could be predicted. No difference between mitosis in the oogonium and in the antheridium could be observed. At the time of the differentiation of the oosphere the contents of the antheridial tube stain deeply, but it has not been possible to demonstrate nuclei in its interior at this age. When the nuclei line up preparatory to zonation the antheridial tube has usually penetrated the periplasm almost to the outer boundary of the oosphere, and as it later pushes into the oosphere, during the telophase of the mitosis, it somewhat indents the boundary film. One oogonium was seen that had two antheridial tubes penetrating the periplasm from opposite sides, but neither had yet pierced the boundary of the oosphere. Another stage was observed where an unemptied tube lay tangential to the oosphere, being apparently the unfavored of two competitors. Still another case presented a stage of fertilization in which two tubes were simultaneously opening into one oosphere.

Minute globules of brownish color are to be seen in the cytoplasm of the oogonium from its earliest development up to zonation. In all early stages they are indefinite in number and irregular in size, but seldom larger than one of the nuclei

When the ooplasm is first differentiated it possesses a small number of these globules which are irregularly distributed. At this time and in earlier conditions there is nothing to indicate that these structures have any peculiar significance. In their color and form they suggest minute drops of some oil-like substance that has remained undissolved under all the treatment experienced by the preparation. However, at the time of zonation, and in certain later stages, there is only one such drop or globule, where there were previously several, and that one is always in the geometrical center of the oosphere, and is surrounded by a differentiated region of cytoplasm (*figs. 69–71, 74*). Whether or not this one central globule, which is a constant feature of the oosphere from zonation until just before fertilization, is developed by a fusion of the drops previously present could not be determined with certainty. This is strongly suggested, however, by appearances like those noted in *fig. 75*, which was drawn from a young oosphere, and seems to indicate that several minute drops were fusing to form the central globule.

As the oosphere matures the central globule remains unchanged, its constancy in size being very remarkable; but the cytoplasm immediately surrounding it becomes more dense, although still shading away gradually toward the outside (*figs. 41, 69, 71, 74*). In all stages observed this globule was surrounded by the remarkable region of dense cytoplasm which differs from ordinary ooplasm in that it stains more darkly and contains fewer vacuoles and nuclei. When the whole central structure is at its maximum development it is a very conspicuous object within the oosphere (*figs. 41, 71*). There occurs also further slight differentiation of the cytoplasm in the immediate vicinity of the globule where the stain is taken more faintly or has a yellowish tint. This inner region also shows a radiate structure under the low power (*fig. 69*), but the highest magnification, such as that employed in drawing *fig. 71* (3300 diameters), failed to demonstrate definite fibers. *Fig. 71* shows in detail this peculiar region of the oosphere; outside is the vacuolate ooplasm, then comes the region of dense cytoplasm with few vacuoles, and finally in the center is the opaque globule immediately surrounded by a lightly stained zone. After reaching the condition of maximum development the structure rapidly loses character, disappearing entirely just before the antheridial tube discharges its contents.

A summary of the history of the central structure may be given as follows: it first appears in zonation, and reaches its maximum development when the daughter nuclei of the first mitosis pass into the oosphere; after that it rapidly degenerates although traces of its presence sometimes persist nearly to the time of fertilization. A body of apparently similar nature was mentioned by Wager as occurring in the oosphere of *A. candidus* and my own as yet incomplete observations on that species indicate that the body seen in *A. candidus* and the central globule of *A. Bliti* are homologous structures, although they differ much in certain details. I find also a structure very like the one above described in the oosphere of *A. Tragopogonis* and *A. Portulacæ* and believe that we have here an organ of the oosphere, perhaps regularly present in the whole genus Albugo, if not in the oospheres of other related genera. It appears with such constancy at certain important stages in the life history of the species, and passes through such a definite course of development that its presence seems to be of importance. May it not be an organ of the coenocytic oosphere?[1]

It should be called to mind in this connection that Dangeard noted in each of the numerous oospheres, in certain species of the Saprolegniaceæ and Peronsporeæ, a central body which appeared just before fertilization. Various interpretations have been given to such structures by different writers, early observers mistaking it for a nucleus. Dangeard supposed it to be the [illegible] but Wager thought that Dangeard was probably mistaken, and that the structure is truly a central body such as he himself found in *A. candidus*. The descriptions and time of appearance make it seem quite possible that the body noted in the Saprolegniaceæ may be homologized with the central globule of the oosphere of Albugo.

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[1] MR. SWINGLE expressed such views at the meeting of the Society for Plant Morphology and Physiology at Ithaca, December 1897.

It is premature to discuss the function of this body until its relations in other species have been closely studied. As described by Wager it appears to be intimately connected with the behavior of the sexual nuclei; but there appears to be no such relation in *A. Bliti*, where its function is perhaps that of an organizer in the oosphere. The body first appears when the oosphere is well defined and is most highly differentiated during the entrance of the daughter nuclei. At this time also occurs the formation of a thin film of denser protoplasm which definitely bounds the oosphere. A great dynamic change occurs in the oogonium when the ooplasm and periplasm are differentiated and the zone of cytoplasm separating the two regions is formed, and in addition there is that remarkable division of the nuclei in such a manner that approximately fifty daughter nuclei are always cast into the ooplasm. Simultaneous with these activities, existing when they are at their maximum and disappearing when they cease, there is formed this peculiar structure, which is so definite in character and so constantly present that it seems to have some functional importance.

In view of the morphological character and possible physiological value of this central structure to the coenocytic oosphere the writer ventures to propose for it the name *cœnocentrum*. It is necessary here to emphasize the difference in structure between the cœnocentrum and a nucleus. The globule is distinctly not a nucleus, as it is much smaller than any nuclei that were seen, stains differently, and is structureless and unchanging. The cœnocentrum cannot be a nucleus with the globule as a nucleolus, since these masses fail to have the internal structure of a nucleus or its limiting membrane. There is no definite demarcation from the surrounding ooplasm, as is plainly shown in *figs. 41, 71, 74, 75.*

In *fig. 75* other structures are shown, the nature of which is unknown. They are small granules which lie profusely distributed in the cytoplasmic strands in all ages of the oogonium, but are particularly noticeable in the fine meshed area of the oosphere. They do not appear when stained in the ordinary way by Flemming's triple stain, but seem always present and conspicuous if stained by Heidenhain's hæmatoxylin, when they are black (*fig. 75*). A hæmatoxylin preparation when bleached and restained for a long time in safranin shows numerous round red bodies in the same position. In appearance they are a trifle longer than broad and often double, as though two were lying end to end, reminding one of large bacilli. It does not seem probable that these coalesce to form the central dot, as might be suggested by *fig. 75*, since they have a different reaction to stain.

SIMULTANEOUS MITOSES IN THE OOGONIUM.

During the differentiation of the oosphere the nuclei in the oogonium divide once (*figs. 58–62, 65–67*), the mitosis occurring about simultaneously for all of the nuclei, cases of independent division of single nuclei never being found. Almost invariably an oogonium in the condition of zonation presents the nuclei in metaphase, or just passing into anaphase (*fig. 65*). In earlier stages, just before zonation, when the cytoplasm is massed in one or several centers, the nuclei are usually in prophase (*figs. 59–62*), but metaphase may be present even as early as the beginning of this process (*fig. 58*). It is apparent that anaphase is never reached until after zonation, and that the mitoses begin when the cytoplasm commences to collect in masses. The nuclear division always occurs when the antheridial tube is in the position shown in *fig. 59*. It would seem, *a priori*, that the differentiation of the oosphere would take place rapidly, since it consists merely in a floating out of the vacuoles and nuclei from the interior region of the oogonium, but mitosis is presumably less rapid. If changed conditions should hasten or retard the process of zonation, one would expect a variation such as does exist in the time correlations between zonation and the mitoses.

The spirem condition of the nucleus has been described, but the other stages of nuclear division have not been considered.

detail. Preparatory to the formation of the spindle the nucleus elongates, its membrane being pulled out in two directions, while the chromatin collects in minute globules in the linin thread. These are at first irregular in size, but gradually become less numerous and more uniform, presumably fusing with one another until a small number of nearly spherical bodies, the chromosomes, are present in the nucleus. *Fig. 4* shows a condition with the chromatin granules and the linin network still evident, while in *fig. 5* the linin strands have almost disappeared. As the chromosomes perfect their organization they approach the equator of the now elongated nucleus (*fig. 6*), and there appear at the poles two round bodies which lie within the nuclear membrane. These bodies stain red with Flemming's triple stain and are constantly present at this period of the prophase. Although not observed earlier they persist and become more prominent in the later stages. The spindle fibers first appear a little later at the poles of the elongated nucleus, from whence they seem to grow toward the equator. They are entirely intranuclear, and there is a distinct space between them and the nuclear membrane (*fig. 9*). The chromosomes are at first irregularly scattered throughout the equatorial region, but when the achromatic figure becomes more distinctly developed they group themselves into a nuclear plate and divide. As the chromosomes are nearly spherical and very small it was impossible to determine the manner of their division. The mature spindle rests in a clear region, distinctly inside of the nuclear membrane, with the polar bodies very definitely outlined. That these bodies must be regarded as centrosomes is evident from their constancy at certain periods of the mitosis, *e. g.*, from late prophase to late anaphase. Being intranuclear, it is not surprising that extranuclear radiations should be absent, and in fact the only radiations present are the spindle fibers. These structures seem not to have been previously described for this group of fungi.

Figs. 7, 8 show a condition very commonly seen. The nuclear membrane is prominent, the chromosomes are massed at the center, and the spindle fibers are very slightly or not at all differentiated. The dark strand shown in the lower half of *fig. 8* is probably like the less conspicuous one in a similar position in *fig. 5*. Both may be considered as the remains of a spirem thread, such as is shown in *fig. 4*. *Fig. 7* is open to a similar explanation. The difference in the shape of these two figures is noteworthy, since it is probably due to their position in the oogonium. The spindle shown in *fig. 8* lay in a strand of periplasm which supported an oosphere, similar to the strands shown in *fig. 62*. It is probable that the length of the spindle is due to the tension to which it was subjected. *Fig. 7* was from a crowded bunch of nuclei, and could not elongate. The appearance shown in this figure might tend to support the idea that the spindle fibers are formed from the linin thread, a view entertained by Wager, but disputed by Berlese. The question presents so many difficulties that the writer does not feel warranted in expressing an opinion.

The nucleolus at the time of late prophase is sometimes small, but often quite as large as when the nucleus is in the spirem condition. It may be found throughout all stages of the mitoses. *Fig. 12* shows the splitting of the chromosomes, and *fig. 13* may be recognized as a condition immediately later; the membrane is still intact and encloses the nucleolus which is outside of the spindle, and the centrosomes are at their maximum definiteness. It is interesting to note in passing that the two nuclei lying very near to the antheridial tube are usually nearly a full phase in advance of other oogonial nuclei in mitoses, a fact strikingly apparent when the majority of the nuclei are in metaphase.

The chromosomes, after the division of the nuclear plate, move poleward with unequal rapidity, the poles lose their aster character, and the nuclear membrane is no longer visible, the boundary of the nucleus being marked by the spindle fibers (*fig. 14*). With the loss of the membrane the whole nuclear structure assumes, and retains through later stages, the property of staining more darkly, a character particularly noticeable in the regions where the chromosomes lie (*figs. 14–18*).

The nucleolus may travel poleward with one group of chromosomes, or break into two, either at this time or earlier, thus allowing a small nucleolus to go to either pole. It is easily distinguished from the chromosomes by stain reaction and usually also by its size. At this stage there is at each apex a round body of the size and shape of the centrosomes, but scarcely distinguishable from the chromosomes except through position. Even in very late anaphase faint fibers may be seen connecting the daughter nuclei (*figs. 15, 16*). When the chromosomes reach the end of the spindle they become indistinguishably mingled and massed (*fig. 16*), but the nucleolus often stands out very distinctly by virtue of its color and size.

After the two groups of chromosomes are sufficiently separated the spindle fibers collapse in the middle (*fig. 18*), and the daughter nuclei become distinctly organized. Each rounds off and contains a dark somewhat crescent-shaped mass of chromatin on the side that is turned away from its sister nucleus. This condition is often very noticeable in the differentiated oosphere when several daughter nuclei may be observed, each with its dark half centerward. The explanation of this condition is not far to seek. The sister nuclei lie in the periplasm with their dark half turned outward, plainly showing that the former mitotic figure lay across the line that separated the ooplasm from the periplasm. These conditions present strong evidence of the source of the nuclei in the oosphere.

Whether or not the centrosome of Albugo persists as a permanent organ of the cell is a question that as yet is impossible to answer. The structure so prominent at metaphase is not seen in the resting nucleus; the conditions, however, are such that it might well exist hidden among the chromatin granules and pass unnoticed. It is so small and its stain reaction so uncertain that negative evidence is valueless.

The shape of the spindle figure may be greatly modified by the conditions; for example, if the nuclei happen to be in prophase or metaphase when the centripetal rush of cytoplasm occurs the tension due to the movement of the protoplasm seems to act conjointly with the normal elongating forces, thus producing extraordinarily long spindles (*fig. 62*). On the contrary, if the nuclei reach the border of the central mass in an earlier stage of mitosis no such forces obtain. Spindles caught in the first massing of the cytoplasm are often distorted and bent like the letter *f*, owing undoubtedly to torsion caused by the vacuoles as they move outward.

MATURATION OF THE COMPOUND OOSPHERE AND OF THE ANTHERIDIUM.

The multinucleate or compound oosphere when completely differentiated contains by actual count an average of [illegible] nuclei. These are found in various conditions of reorganization following the mitosis at zonation, and they rapidly assume the typical condition of a resting nucleus, each showing a prominent nucleolus and very faint linin network. A nuclear membrane is sharply differentiated. Presently the linin network becomes more prominent and a spirem condition is reached, very like that first observed in the oogonium. A mitosis now occurs in the oosphere affecting all of its nuclei, and is similar in all important details to that just described for the oogonium as illustrated in *figs. 22–30*. The nuclear figure stains much more faintly than that of the previous division, the spirem appearing lighter and skeleton-like in comparison with that of the first mitoses. The only other important differences noticeable are in the more pointed anaphase and telophase figures. Compare *figs. 28–30* with *figs. 14–18*. The spindles are always long (*fig. 30*), and this fact renders it easy to detect the formation of the new membranes around the daughter nuclei by the collapsing spindle fibers. The daughter nuclei round off, pass into a resting condition, and are ready for fertilization.

While this division proceeds in the oosphere a similar mitosis occurs in the antheridium. Since the antheridial nuclei divide simultaneously with the oogonial nuclei, passing into the resting condition, and are found in mitosis when those of the oosphere divide, it is evident that they undergo two divisions. It is in esting to note that the two nuclear divisions in the antheridium and in the oogonium are similar in character and proceed simultaneously. The antheridial tube at the time of the differentiation of the oosphere lies in the periplasm, with its apex close to the bounding film of the oosphere. It now pushes into the oosphere, increasing in diameter as it advances. It takes safranin stain greedily from this time until it discharges its contents, but if the stain be thoroughly extracted in acid alcohol and the preparation treated with gentian violet the contents become clear. The tube when fully developed is seen to contain numerous nuclei. A glance at *figs. 73, 76* will give a clear notion of this condition. It will be seen that many nuclei are massed near the tip of the tube and that others are apparently entering at the base. It is impossible to determine their number by actual count, owing to the crowded condition (*figs. 77, 85*, etc.). However, as there are about 35 nuclei originally present in an antheridium, and these divide twice, there must be altogether about 140. Of these, 20 or 30 perhaps remain in the antheridium proper, leaving a little more than 100 to pass into the tube. The antheridial tube pushes toward the center of the oosphere during the second mitosis (*fig. 70*), and arriving nearly at the center its tip swells, becoming nearly globular. In this condition the end of the tube is covered by a very thin wall which is barely visible, and yet holds within a dense mass of sperm nuclei (*fig. 77*).

When the male nuclei enter the antheridial tube they possess the characters of resting nuclei, but as they approach the tip they become oval, and later pointed at both ends, and the anterior end is seen to contain the nucleolus around which is massed a densely staining substance, probably chromatin. *Figs. 31, 32* shows nuclei from both the base and tip of the same tube, that which is illustrated in *fig. 73*. In the narrow entrance and basal portion of the tube the nuclei are necessarily arranged in single file, but as its diameter enlarges they become massed in dense groups, and the tip is so closely packed with nuclei that it reminds one forcibly of the appearance of a raspberry with its drupelets (*fig.* 77). Two sections of the same antheridial tube are shown in *figs.* 77, 78, one at the tip showing numerous nuclei surrounded by a very delicate membrane, the other near the base giving a view of the narrow nearly empty cavity and the thick wall. As the tube enlarges the protoplasm in the antheridium proper becomes more and more vacuolate, but its contents never entirely leave the structure (*figs.* 80*a*, 86). The film separating ooplasm from periplasm is but slightly if at all changed by the entrance of the antheridial tube and during the maturation of the oosphere. The periplasm likewise shows no important changes. Some of its nuclei divide mitotically, but the number does not seem to increase materially. Most of them remain in a resting condition. One case was observed where every nucleus in the periplasm was undergoing mitotic division simultaneously with those of the oosphere, but this must be regarded as a very exceptional instance.

FERTILIZATION.

The conditions are now ripe for the act of fertilization. The female nuclei resulting from the mitosis in the oosphere, about 100 in number, are in resting condition. The antheridial tube is filled by an approximately equal number of male nuclei, and its tip has swollen so that the contents are separated from the ooplasm by only the thinnest of walls. The wall finally vanishes and the contents of the tube are free to mingle with the cytoplasm of the oosphere (*figs.* 80, 82). The sperm nuclei move through the ooplasm toward the female nuclei, their way being often marked by a streak of denser cytoplasm. There is no visible cause of this movement, but as the male and female pronuclei differ in form a chemotropic influence may perhaps be safely inferred. Longitudinal sections of antheridial tubes (*figs.* 80, 82) sometimes show the nuclei pouring out, and transverse or oblique sections (*figs.* 84, 85, 86, 77, 78) corroborate the proof of a discharge of many nuclei. There is in all of these sections unmistakable evidence of a multinucleate discharge from the antheridium into a multinucleate oosphere. Sections of the antheridial tube similar to those figured, both transverse and longitudinal, are not uncommon in the writer's preparations, and many have been carefully studied. No antheridial tube was found which gave any evidence of the possibility of the discharge of but one functional sperm nucleus.

When the sperm nuclei emerge from the tube their nucleoli are in the anterior ends, and later there appears prominently in the same region a substance that stains like chromatin. As the sperm nucleus approaches the female nucleus a faint linin network becomes visible (*figs. 33, 34, 35*). When the sex nuclei first come in contact the male is the smaller, but later they become approximately equal in size. It seems probable that the female nucleus actually decreases slightly in size during this equalization. The nuclei do not immediately fuse, though both are apparently in resting condition. All stages of fusion can be easily observed. The sexual nuclei are pressed together, the sperm nucleus first assuming a spherical form, the bounding membrane disappears at the point of contact, and there results one dumb-bell-shaped nucleus (*figs. 37, 38*). As the coalescence becomes more complete the fusion nucleus takes on a spherical form, and presents the structure of a resting nucleus. No details regarding the fate of the linin network were obtained, owing to the extreme minuteness of these structures and the increased difficulty in staining them in a manner adequate to their study (*figs. 35–40*). Fusion must be a process of extreme slowness, judging from the advance made in other structures of the oospore during its consummation.

A general view of an entire section of an oospore during the pairing of the sexual nuclei is shown in *fig. 88*. A count of the number of pairs in all of the sections of such an oospore gives an average of about 100. There seems to be a slight excess of sperm nuclei, as occasional small unpaired nuclei are found during the fusion stage; there are also several nuclei left in the antheridium proper and in the base of the tube. Sections of the oospore in which the nuclei are fusing present no trace of the antheridial tube inside of its wall, although it is easily traced through the periplasm (*fig. 91*). Judging from this condition the terminal portion of the tube must vanish immediately after giving up its contents. The portion imbedded in the periplasm becomes thickened, resembling the primitive wall; but it seems never to attain the character of the mature epispore, as is the case in so many other species of Albugo. Indeed, no traces of the antheridial tube were ever seen in ripe spores.

The character of the ooplasm changes when the antheridial tube opens. As may be seen by comparing *fig. 70* with *figs.* 8 *82, 84, 85* the vacuoles increase considerably in size and become irregular in form. The most striking feature of this later condition, however, is the tendency of the contents of the oosphere to break away from the periplasm (*fig. 80*), a phenomenon never met in younger stages. This indicates that changes have occurred at the boundary between the ooplasm and periplasm. Indeed, it is at this time that a true wall may be first observed around the oosphere. It will be remembered that previously the periplasm and ooplasm were separated only by the delicate film that appeared during zonation; but now for the first time a distinct wall is present around the ooplasm, and its advent seems to be correlated with the opening of the antheridial tube.

The wall occupies precisely the position of the film between the ooplasm and periplasm, and is probably formed by a further development of that structure. Its intermediate position between the ooplasm and periplasm and the apparent organic connection with both leads to the belief that it is the product of the joint action of both regions, rather than of either ooplasm or periplasm alone. Since this wall remains perfectly distinct from the walls that are formed later, it will hereafter be called the *primitive wall*. This term is used simply for convenience in this paper. Further study of related forms may establish important homologies and lead to further classification. The primitive wall is very clear and homogeneous in structure, entirely without striations, and shows great regularity of curve and thickness. From this time on the condition of the developing walls serve as an index to the age of the oospore.

A stage of somewhat frequent occurrence is shown in *fig. 87*. Judging from the presence of the primitive wall, the character of the ooplasm, and the absence of the antheridial tube, it must follow the opening of the latter, and since the nuclei are not yet paired must precede the condition shown in *fig. 88*. There are two possible explanations for this condition, consisting as it does of an oospore containing several groups of nuclei, each cluster imbedded in a mass of denser cytoplasm. Perhaps these nuclei are gathering the cytoplasm about themselves, a phenomenon of rather frequent occurrence with sexual nuclei; or it may indicate the breaking up of the mass of nuclei and cytoplasm that was released from the antheridial tube. The latter explanation seems more probable. If it be true, a stage similar to that shown in *fig. 88* would result through a further fragmentation of these nucleated masses of denser cytoplasm.

The previous pages have dealt entirely with descriptions of the antheridial tube, the discharge of its multinucleate contents, and the subsequent fusion of sexual nuclei in pairs. For the sake of completeness, and in view of the peculiarity of the conditions and the general, if not universal, belief in a simple process of fertilization, involving only two sexual nuclei, it seems desirable to discuss the possibility of such an occurrence taking place in the oosphere, together with the events already described. A simple fertilization predicates the existence of one female nucleus in the oosphere and one male nucleus in the antheridium; these are either alone, or if with others at least different in structure and function from them. Subsequent to its final differentiation the oosphere never contains less than 40 nuclei. At a later stage both antheridium and oosphere contain about 100 nuclei, but neither contains a single nucleus differing in appearance from the others. If a uninucleated oosphere exists it must be before the oosphere is fully differentiated. A glance over the drawings shows that no such uninucleate stage is represented, nor was there ever the slightest hint of such a condition found during a most persistent search, involving hundreds of oogonia. That such could have existed and escaped observation seems very improbable. The impossibility of the central body either being or containing a nucleus has been sufficiently discussed on a previous page. The search for the single nuclei proved in vain.

The refuge left for an adherent to the idea of a simple fertilization, involving only two sexual nuclei, lies in the assumption that the nuclei of the compound oosphere (*figs. 68, 69*) have descended from a fusion nucleus, which, owing to its rapidity of development may have escaped observation in earlier conditions. That is to say, fertilization might have occurred at a stage similar to that presented in *fig. 64* or earlier. If this were true we would expect to find the ooplasm presenting 2, 4, 8, 16, 32, 64 etc., nuclei, in stages following the division of such a fusion nucleus. As a matter of fact no such conditions were ever observed, or is there the slightest evidence that they could be present. The oosphere when first differentiated contains 40–50 nuclei, derived from the mitotic figures that line up in the manner shown in *figs. 64, 65*. This number is increased to about 100 by the mitoses in the compound oosphere (*fig. 70*), and then comes the observed act of fertilization (*fig. 85*), the discharge from the antheridial tube of a large number of sperm nuclei and the subsequent fusion of these in pairs (*fig. 88*) with the female nuclei. Previous to the act of fertilization the antheridial tube gradually fills with nuclei as it presses deeper into the ooplasm. There is of course a time when the tube contains a single nucleus but this is when it is about one third the size finally reached, and long before it shows any indication of opening.

It is true that very early in oogenesis the dense cytoplasm in the interior of the oogonium may contain a small and very variable number of nuclei, as is shown in *figs. 61, 62, 64*. But there can be no doubt that these conditions represent part of the process of zonation, and they have been discussed in that section of this paper headed "Differentiation of the compound oosphere." It is very probable that stages similar to these might be found where there is only one nucleus left behind in the oosphere in the process of zonation, but the condition of the antheridial tube and all the further history of the oosphere show that this is not the time when an act of fertilization could possibly take place. Moreover, when only one or two nuclei are present they are *always peripheral*, which would not be expected if they resulted by the division of a fusion nucleus. Again, these scattered nuclei are always in the same condition as those near the outside of the developing oosphere, and this is almost invariably a metaphase of mitosis. This coincidence is inexplicable on the basis of their being the result of the division of a fusion nucleus, but it follows as a necessity from the explanation offered in this paper. If they are the product of one nucleus, which has undergone three or four divisions, we have to assume not only the existence of the sex nuclei, their fusion, and the fusion nucleus, but also the resting, prophase, telophase, and anaphase conditions in the formation of 2, 4, 8, 16, and 32-celled stages. An anaphase nucleus is never seen in an oogonium except when the nuclei divide simultaneously either during the first or the second mitosis. The first anaphase always occurs when the general appearance is that shown in *fig. 67*, *i. e.*, when the nuclei are completely lined up and the ooplasm is well differentiated. The second appears in the oosphere after its complete differentiation (*figs. 70, 71*). It is impossible that the 50 nuclei of the oosphere can have been derived from a single hypothetical fusion nucleus.

If attention is turned to the antheridial tube it might be suggested that fertilization could take place at an early period, when the conditions are like those shown in *figs. 62, 64*. But it is only necessary to make plain the fact that the antheridial tube is always very short at this time, and invariably occupies the position shown in *figs. 62, 64*. The wall of the tube is thick, the tube rarely if ever contains nuclei, and there is not the slightest indication that it is at all ready to open. If it be assumed, however, that it does release, in some manner difficult to detect, a single nucleus that is really the male nucleus, and which fuses with the female nucleus, how can the continued growth of the tube and the development of such peculiar conditions as are shown in *figs. 65, 70, 73, 76, 77, 80, 82, 83*, etc., be explained? Why does the tube continue to grow after functioning only to meet the difficulty of disposing of its comparatively massive body and numerous nuclei in the ooplasm? Why do its nuclei later assume a specialized form, resembling sperms (*fig. 85*)?

In considering the positive side of the argument, in favor of a multinucleate fusion, no step is left to be filled by assumption. All of the stages were seen repeatedly and the correlations are perfect. The antheridial tube opens at the culmination of a period of gradual development which has been completely traced. After it has emptied its contents it immediately disappears. The oosphere has likewise passed through a series of remarkable but perfectly graded conditions with all the steps of development clearly shown. Coincident with the opening of the antheridial tube certain marked changes appear in the cytoplasm; the oospore wall is formed, the ooplasm immediately becomes much vacuolate where it was previously dense and uniformly constant in character. The discharge from the antheridial tube introduces into the oosphere a large number of nuclei clearly different in form from those previously there. These sperm nuclei are seen in all positions of exit from the tube, and finally become so distributed as to indicate with certainty that they approach the female nuclei.

At a stage positively older (judging by the development of the primitive wall), the oosphere is found full of fusing pairs of nuclei. That these are not nuclei dividing amitotically is proved by the number of nuclei in the oospore decreasing rather than increasing, and also by the evidence presented through detailed study.

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[*To be concluded.*]

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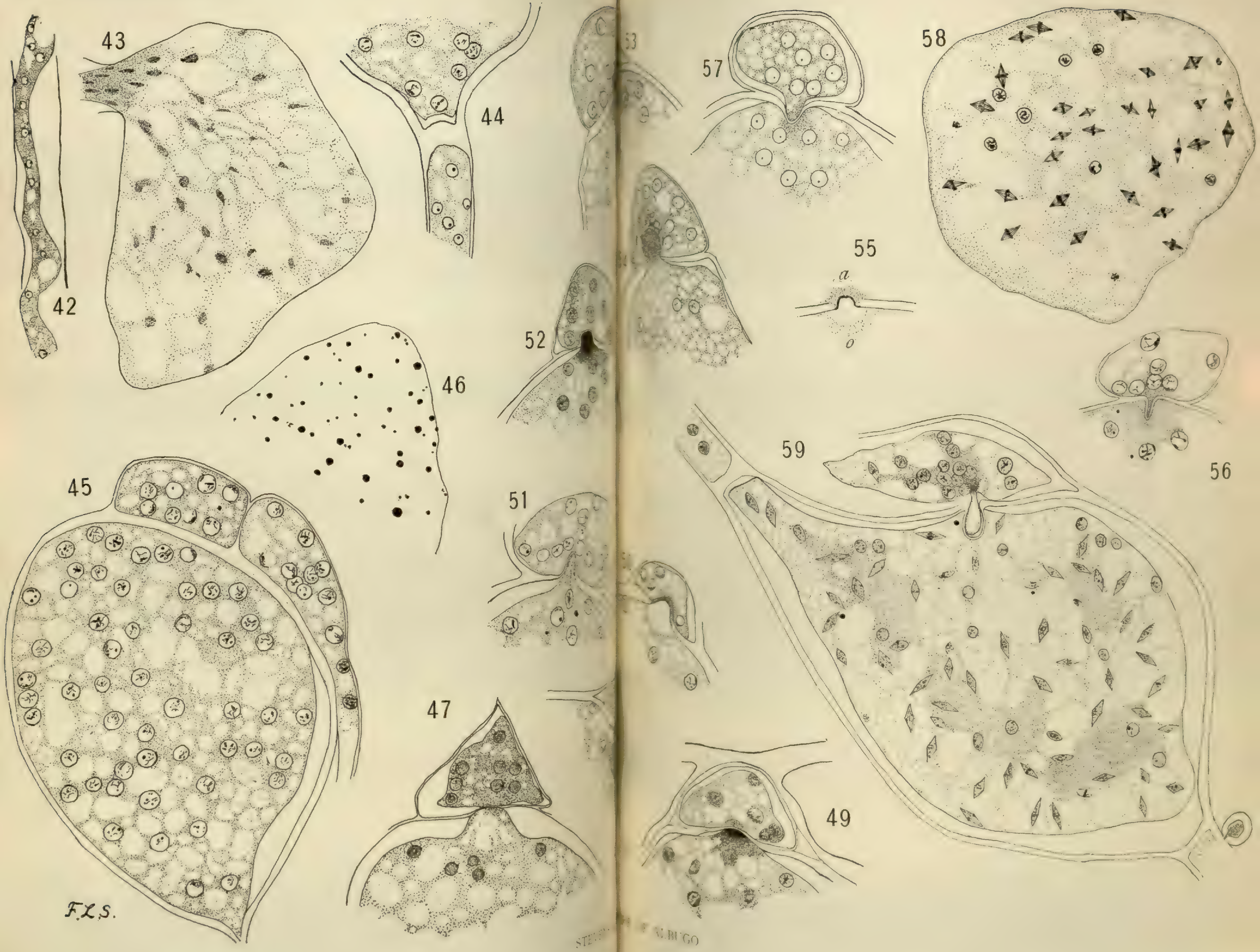
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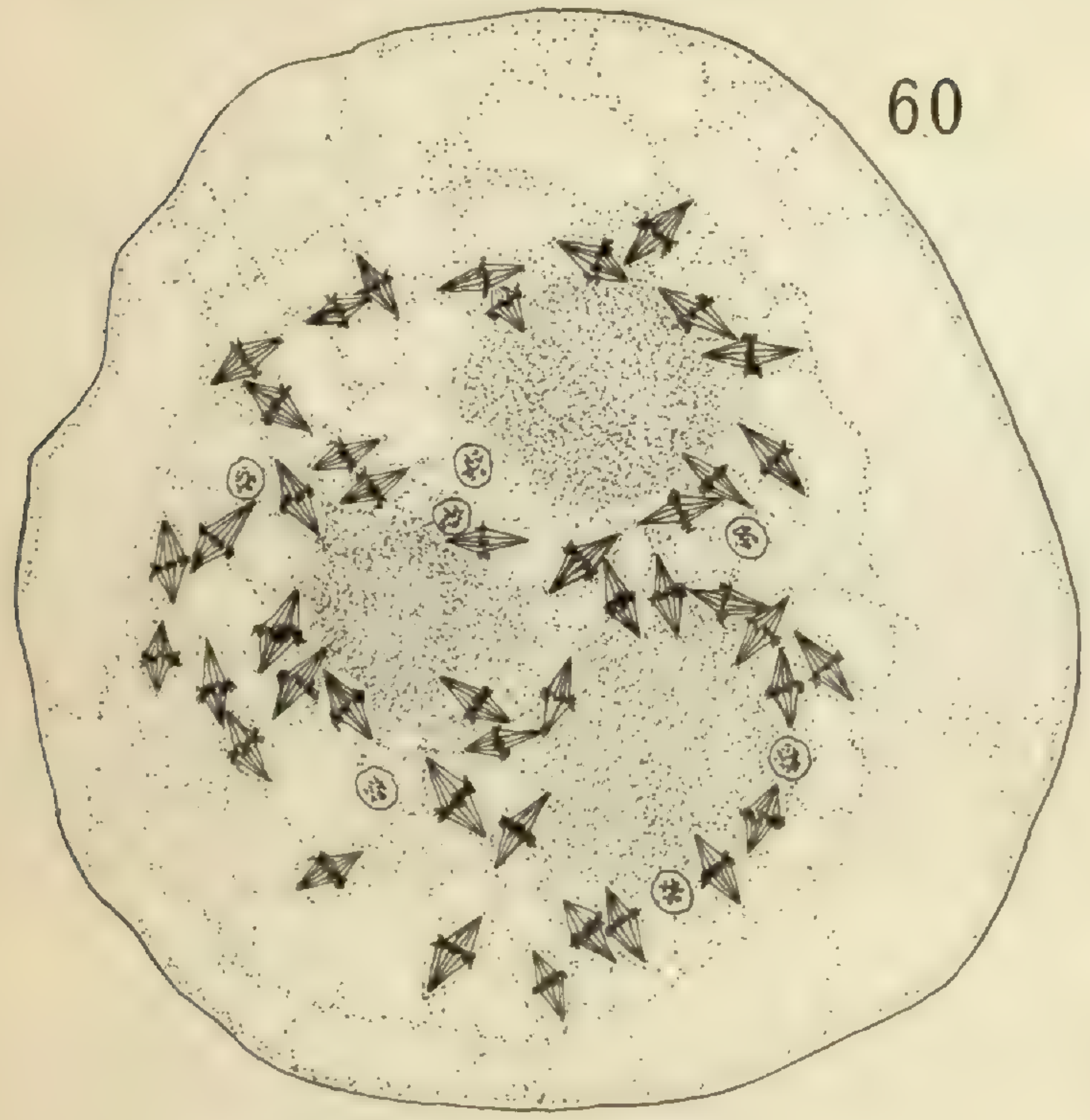

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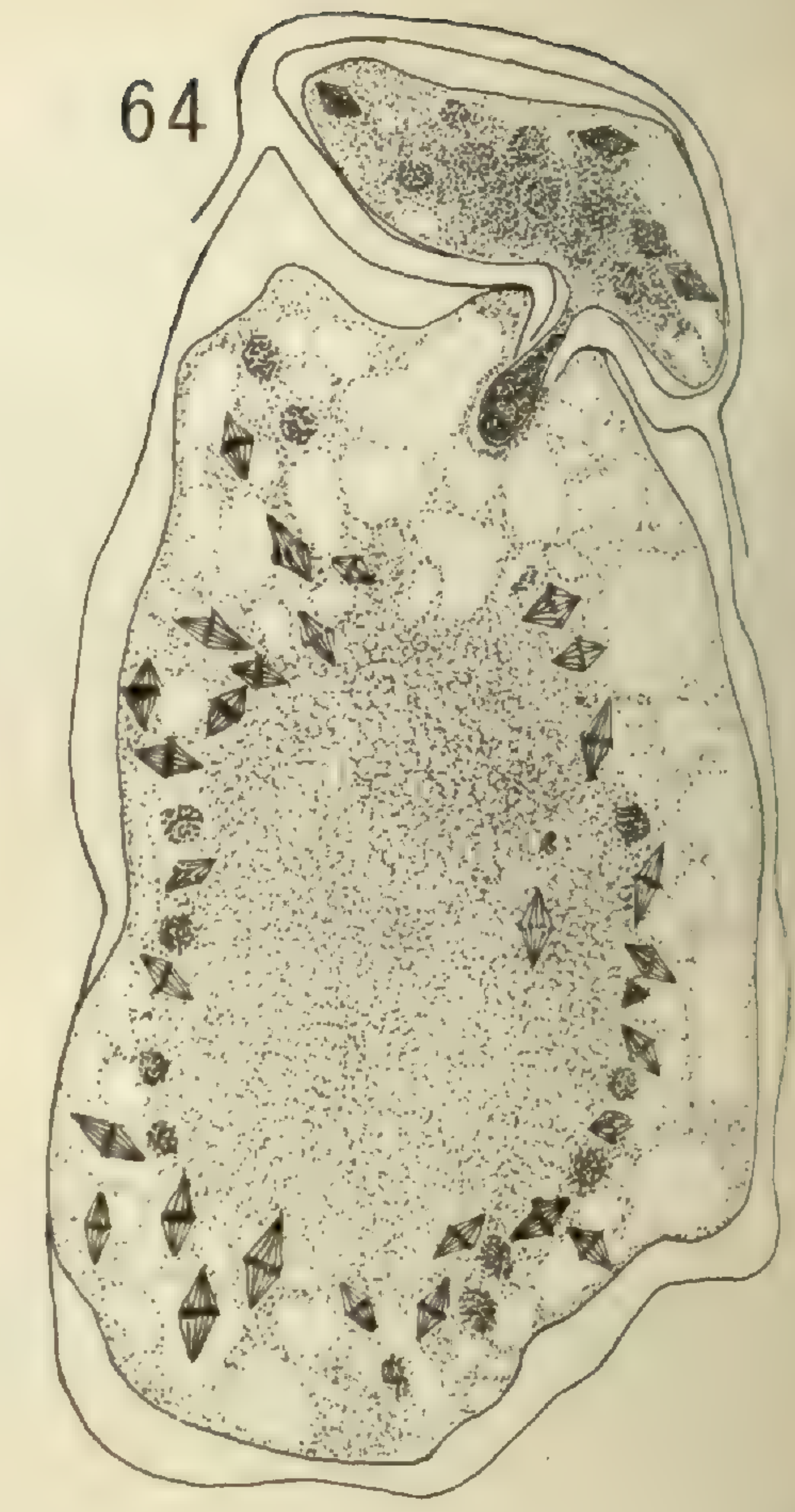

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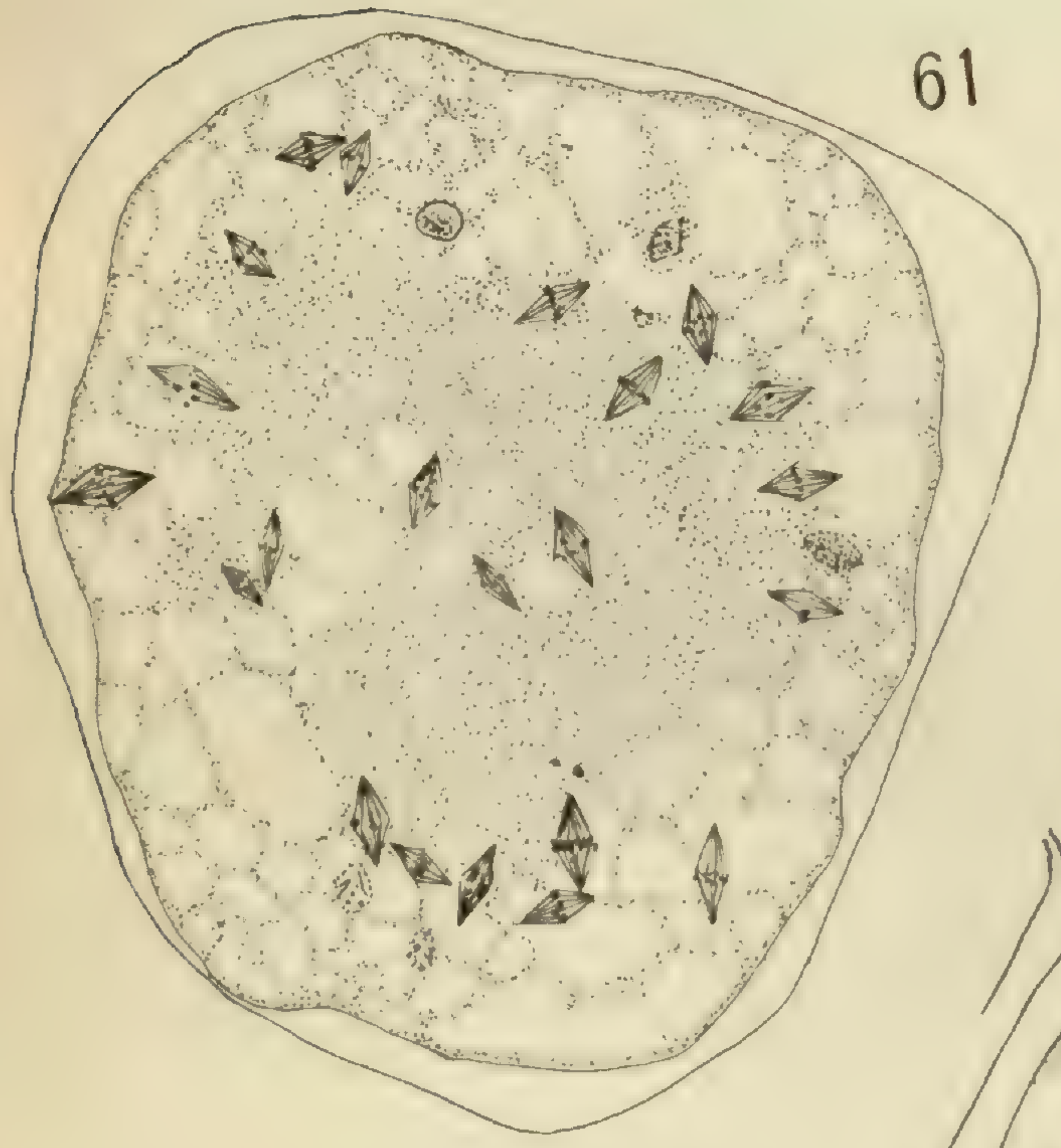

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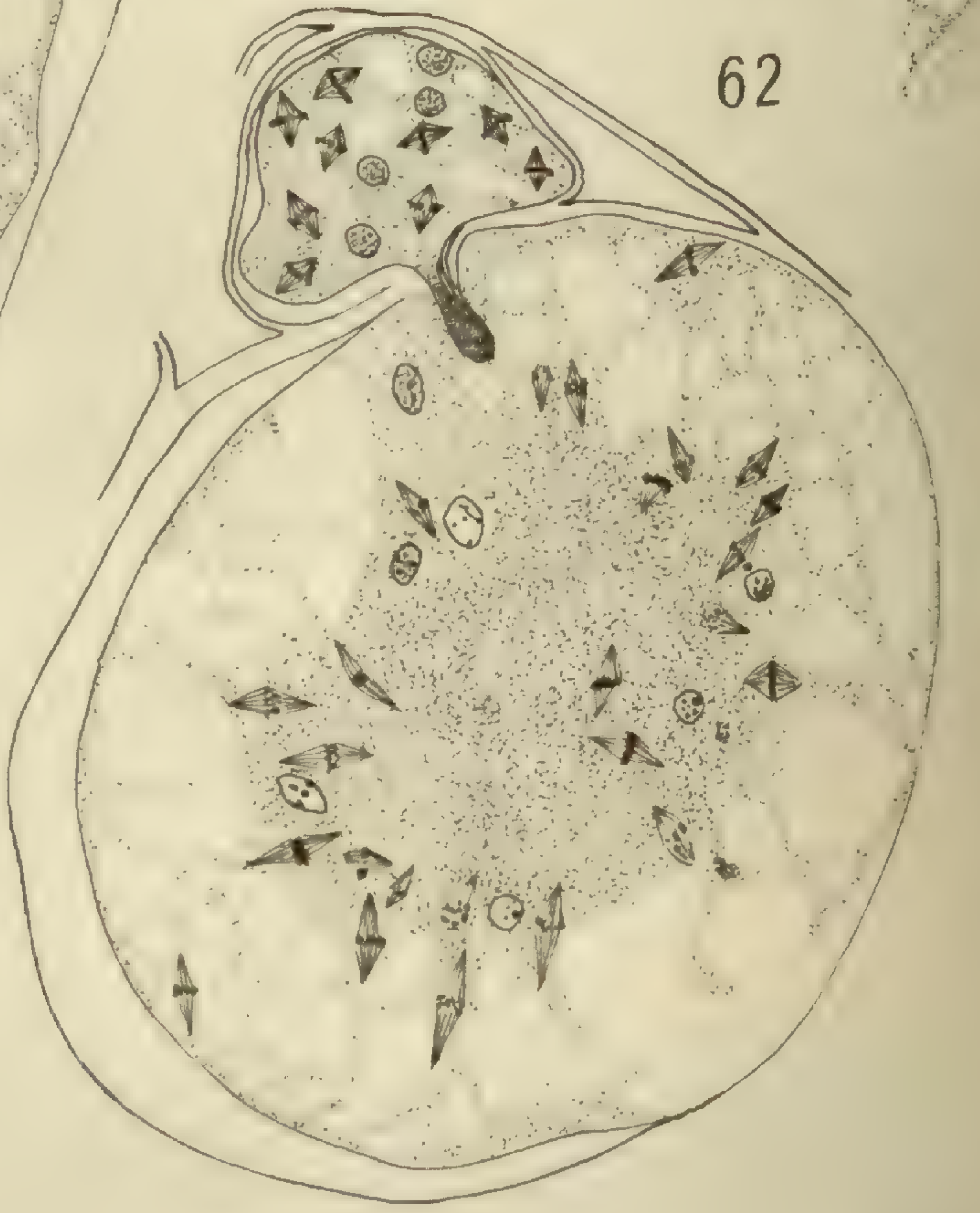

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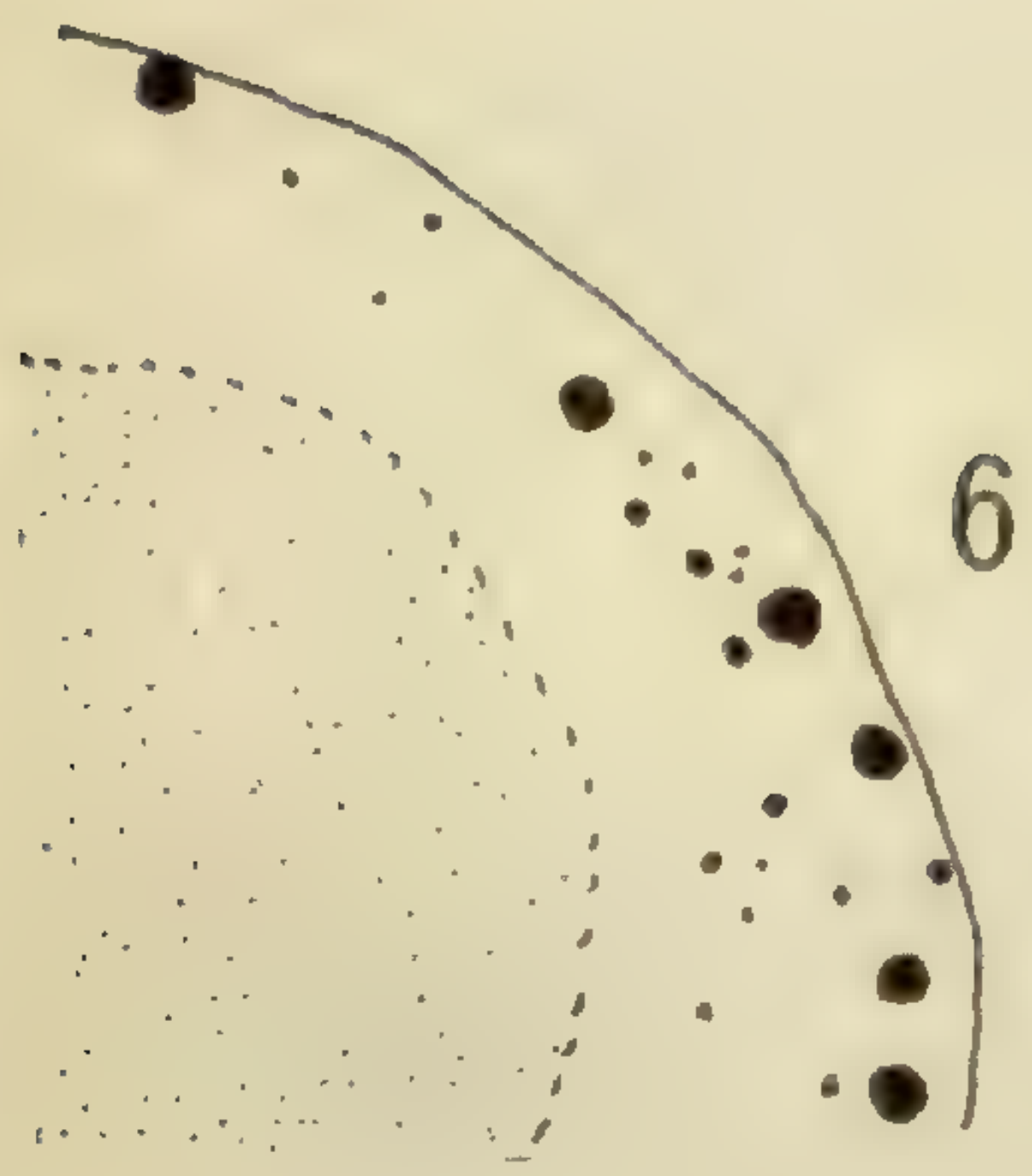

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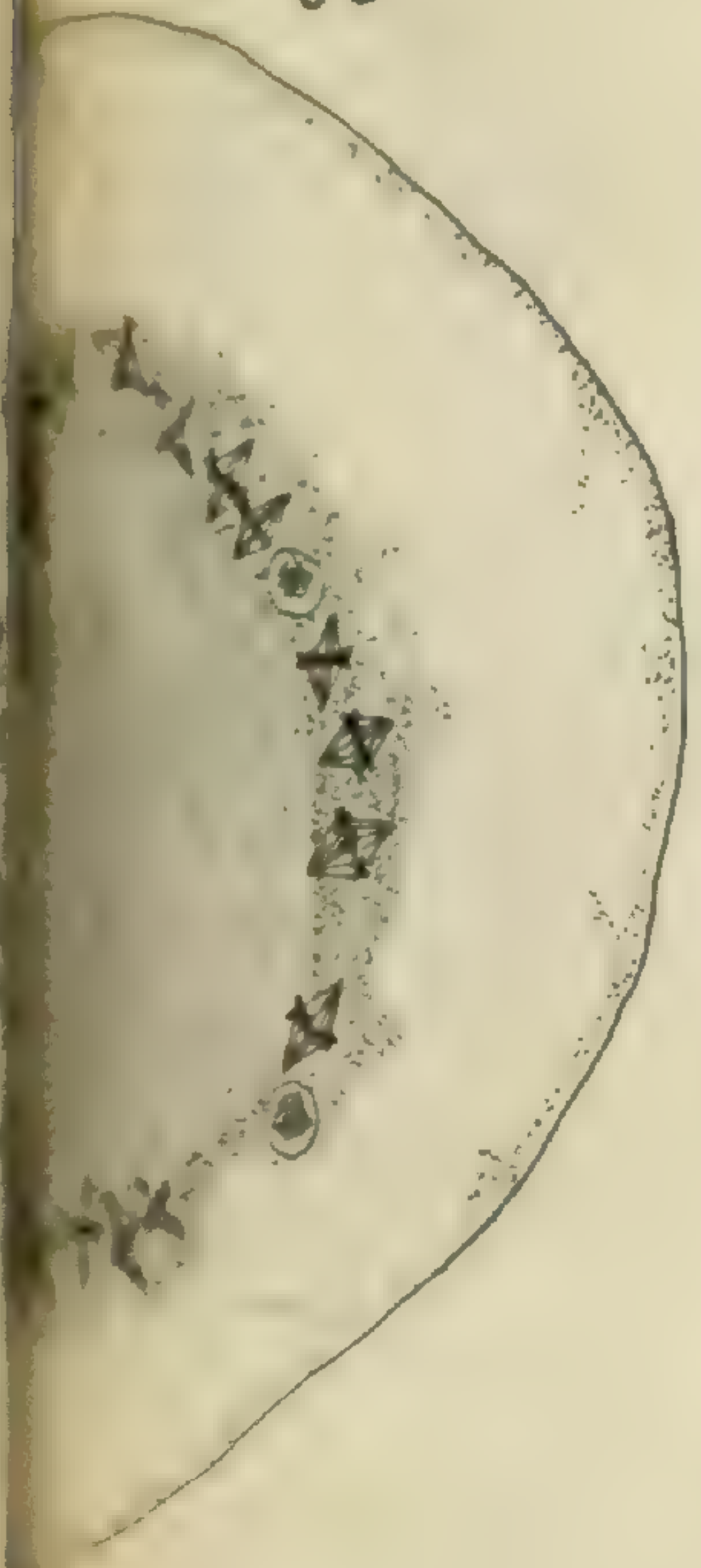

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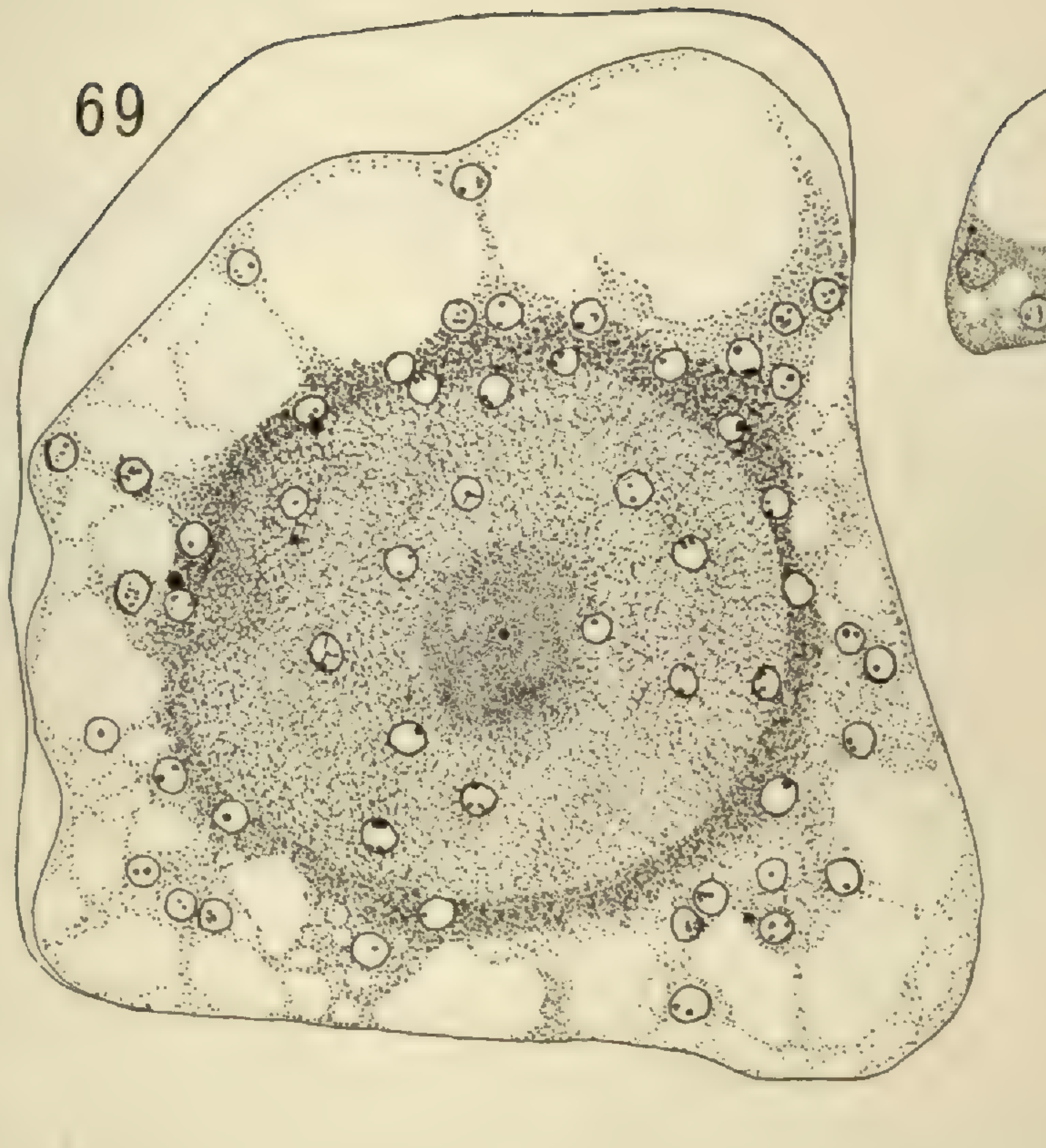

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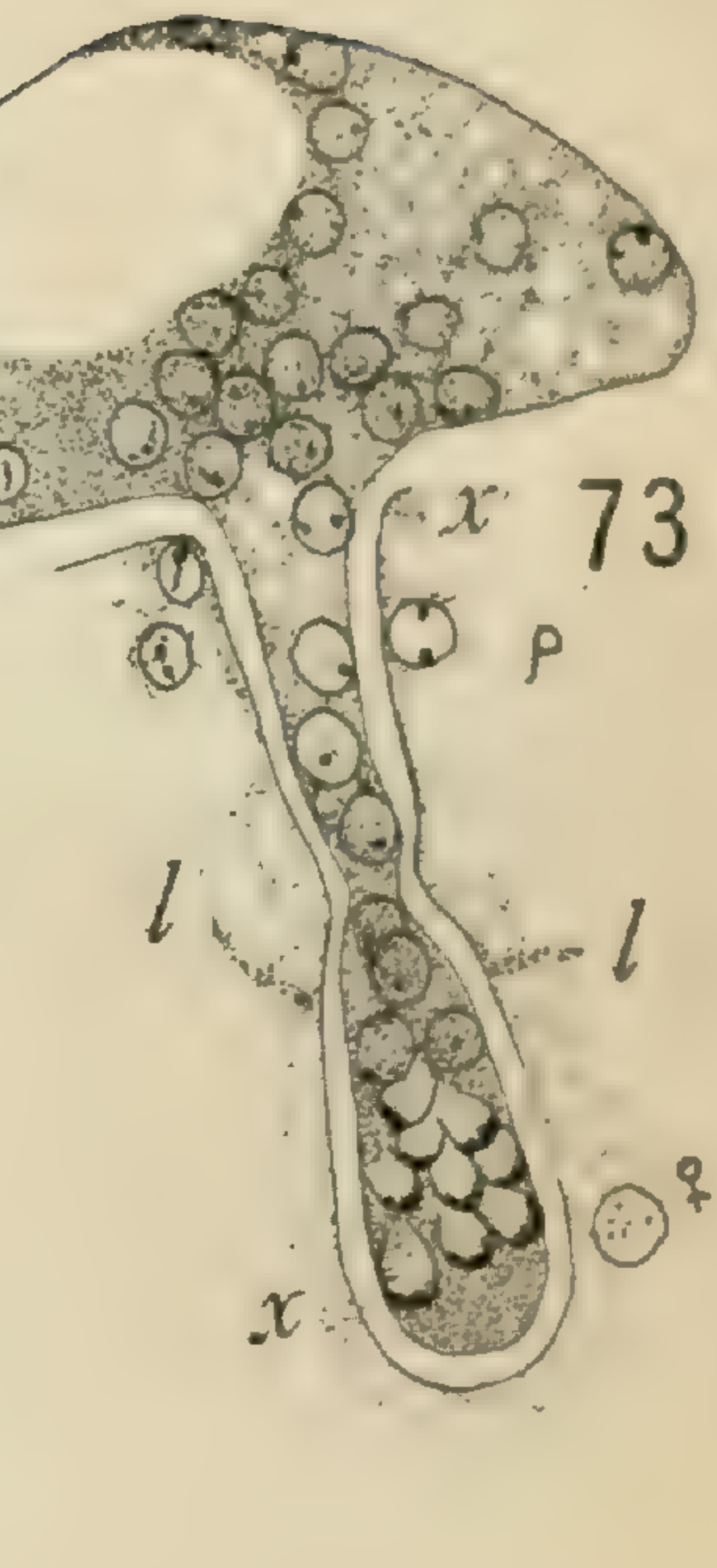

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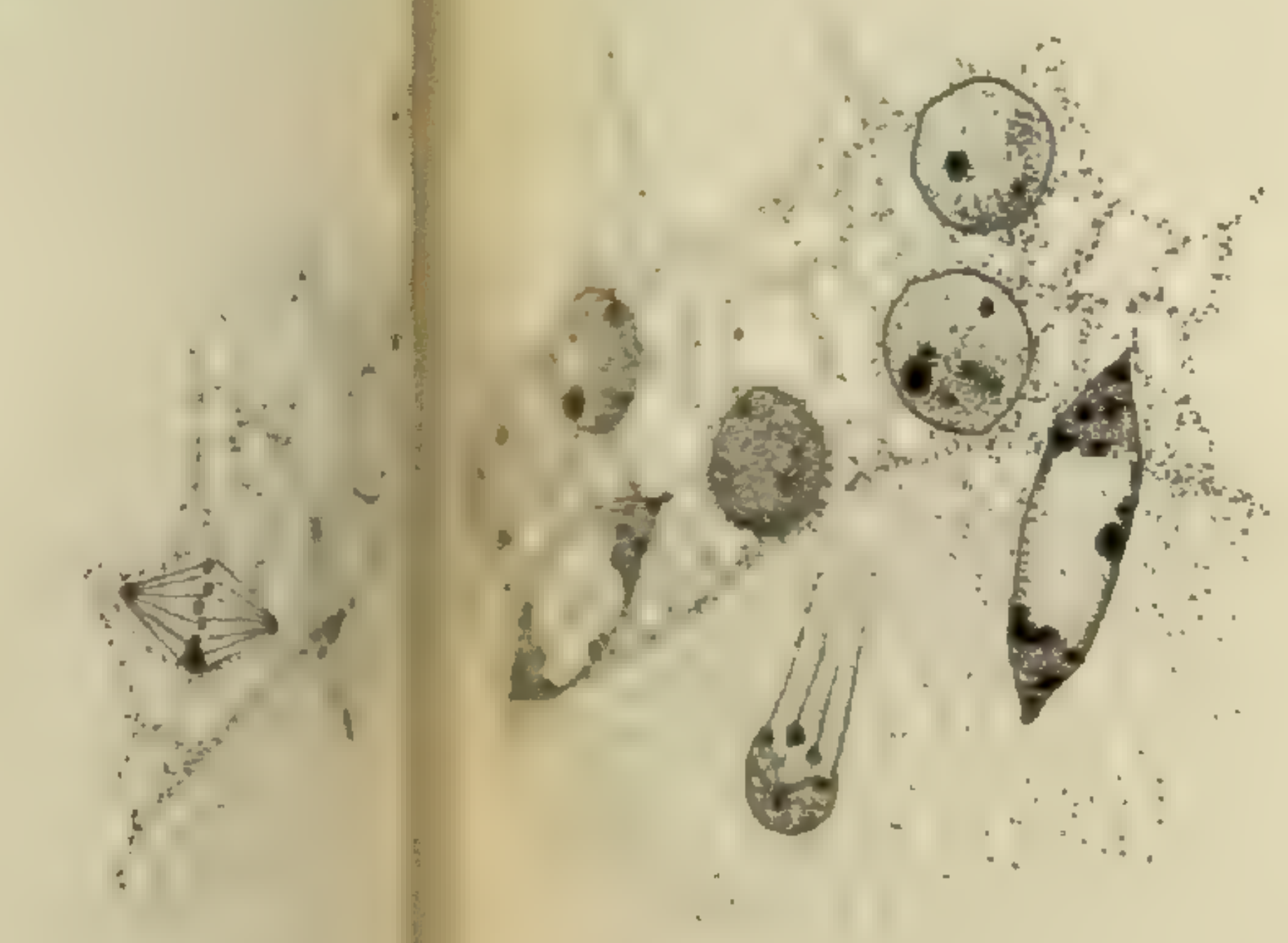

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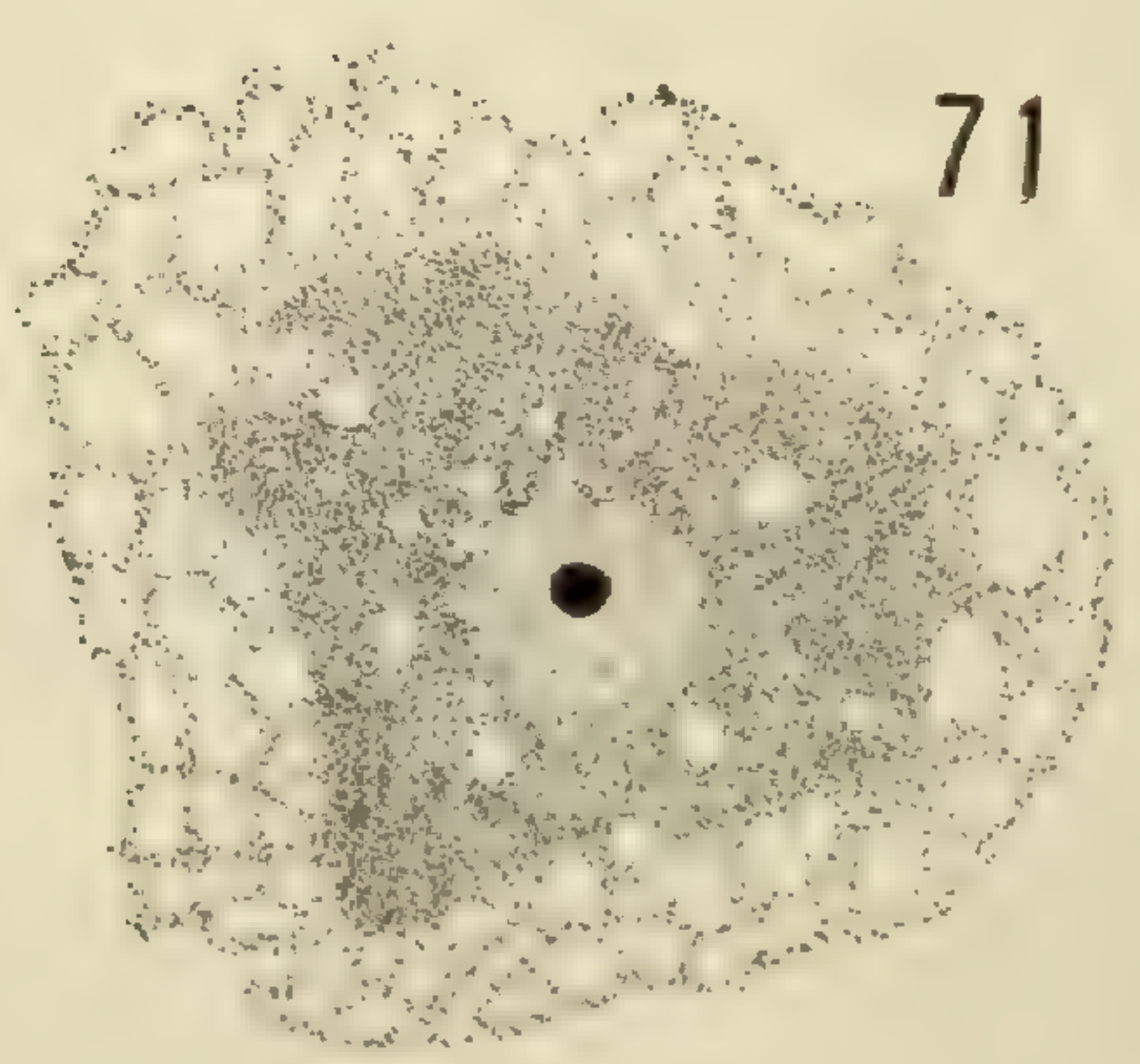

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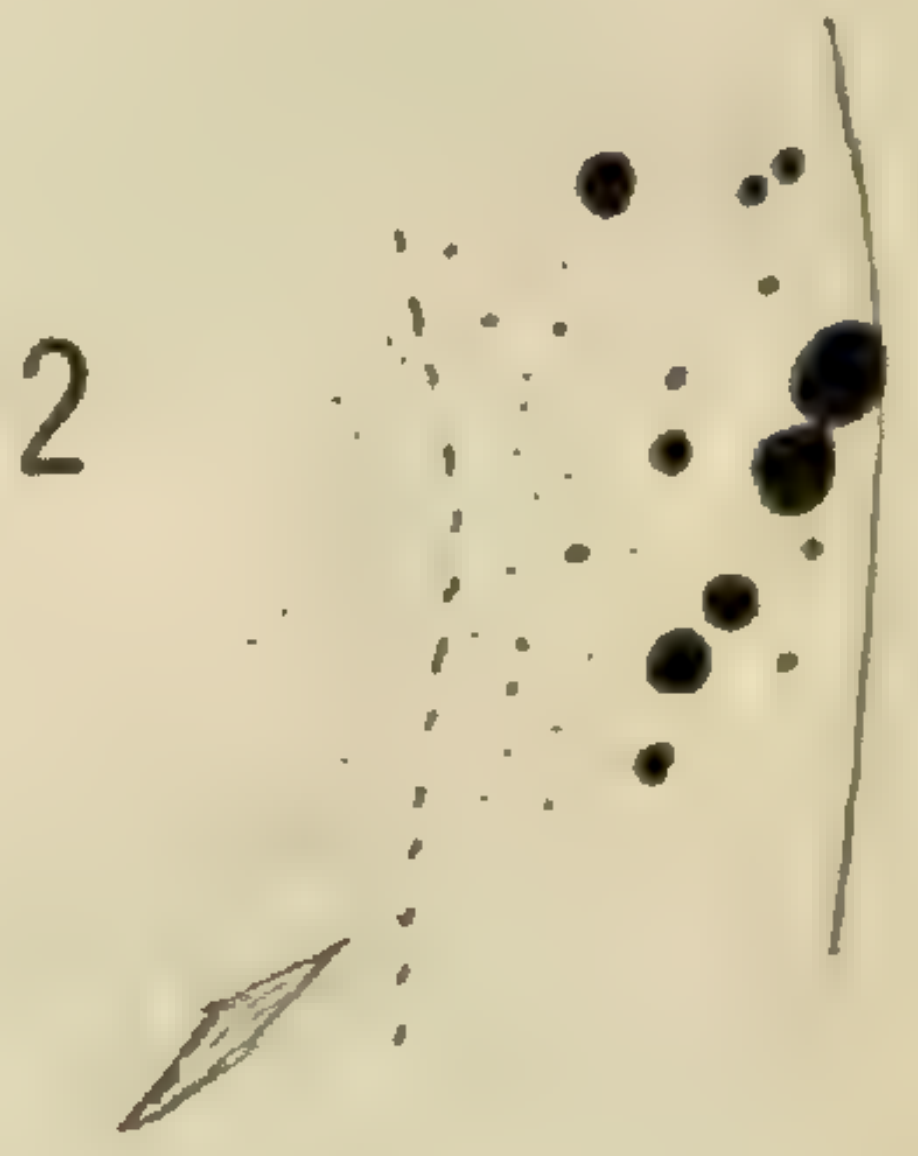

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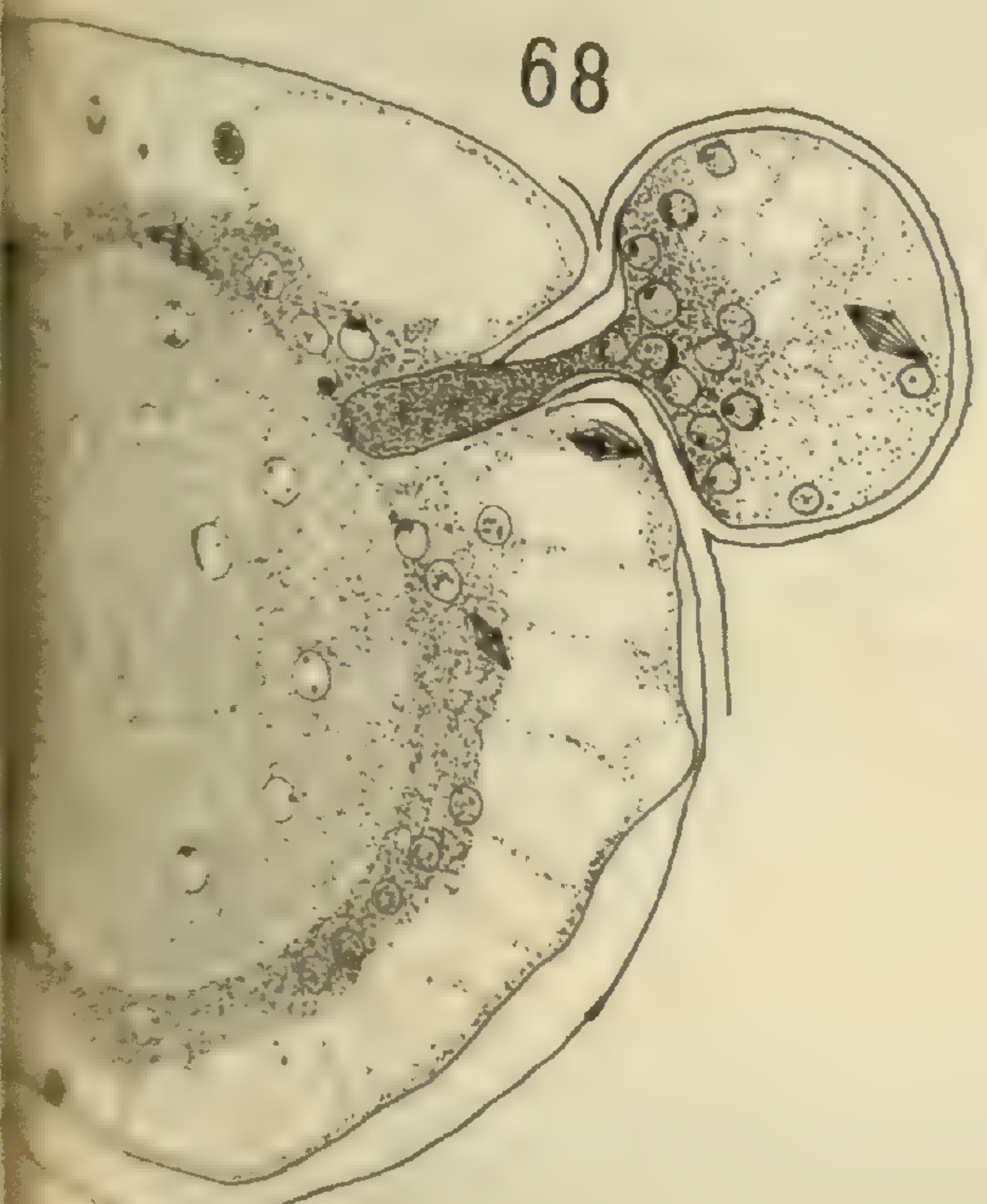

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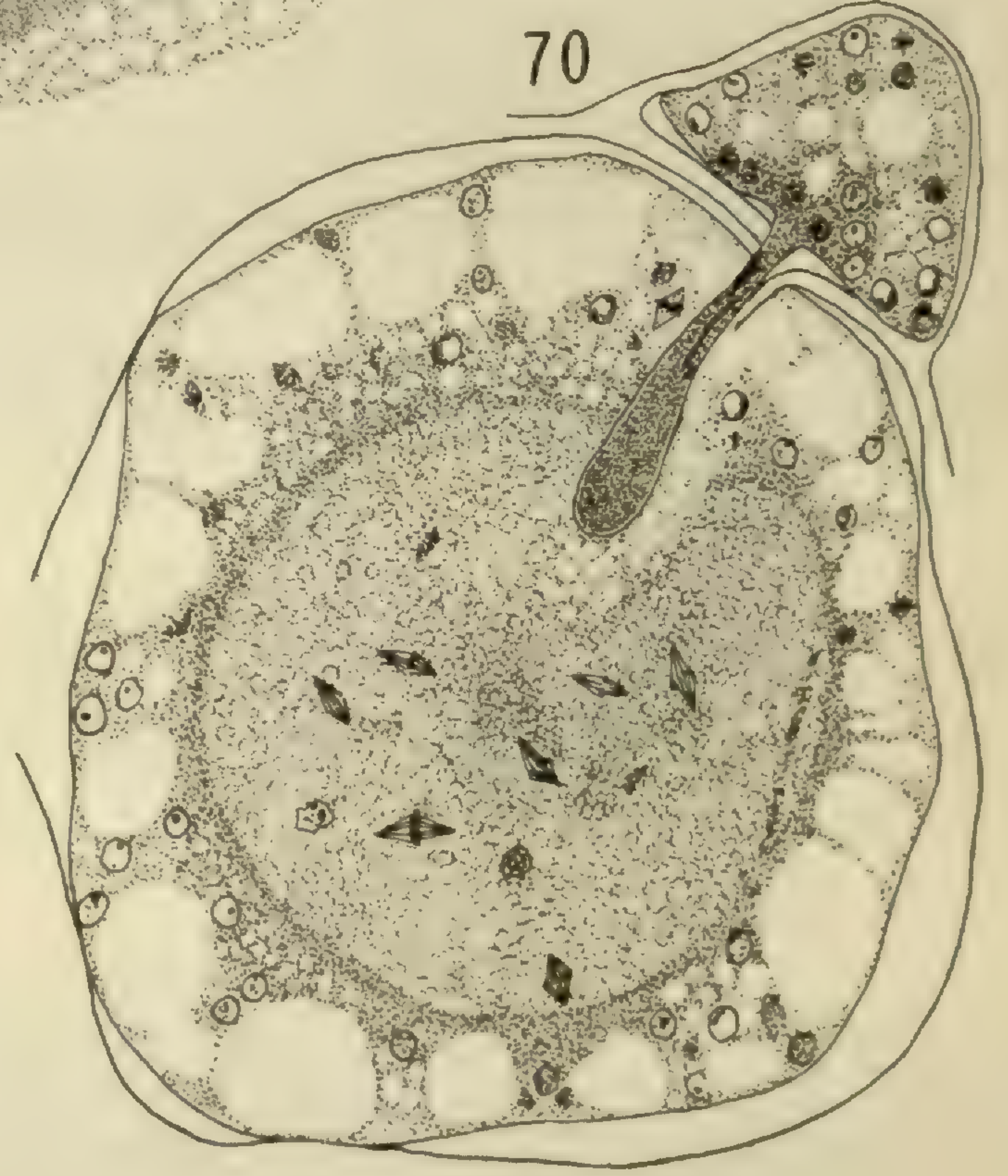

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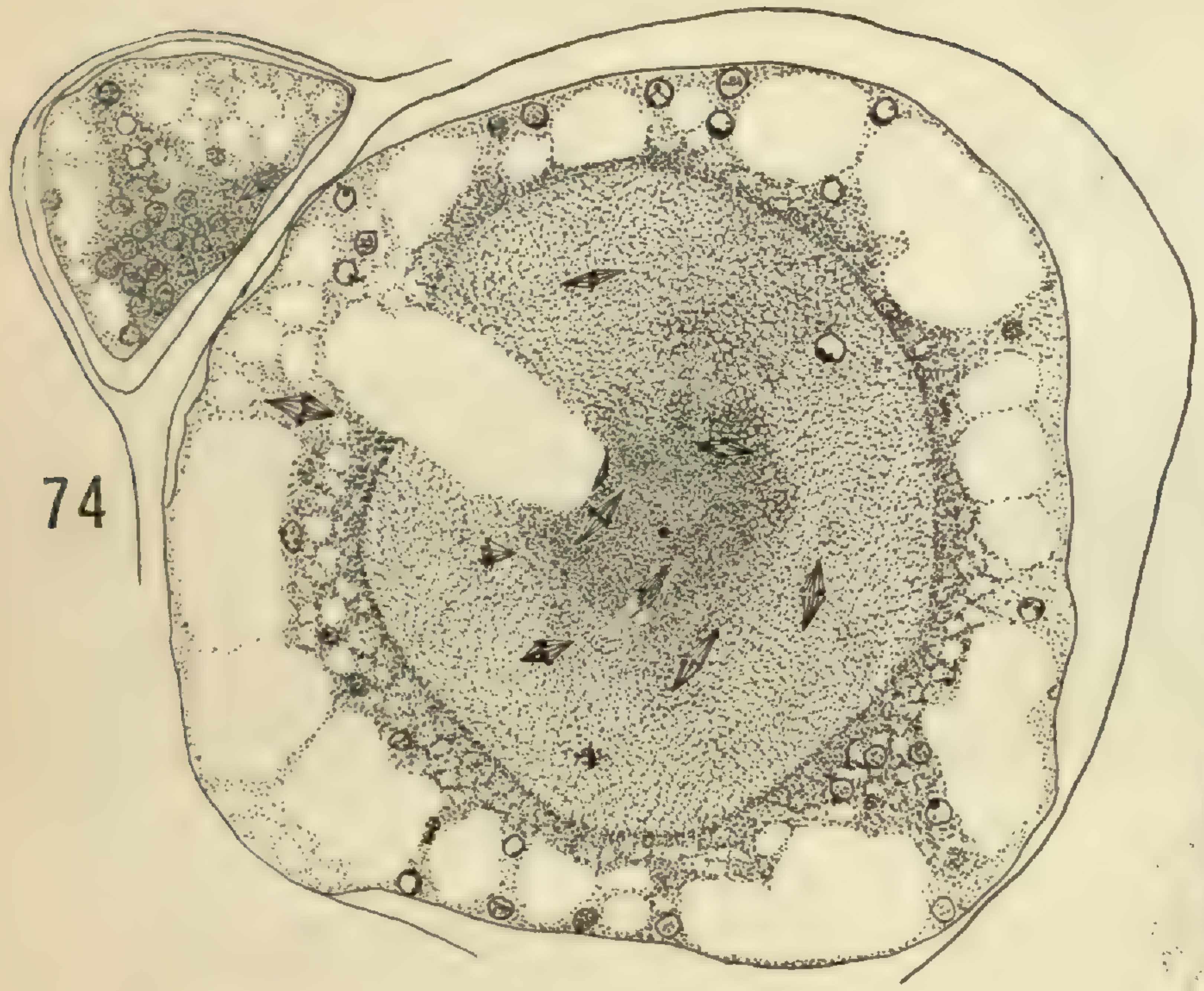
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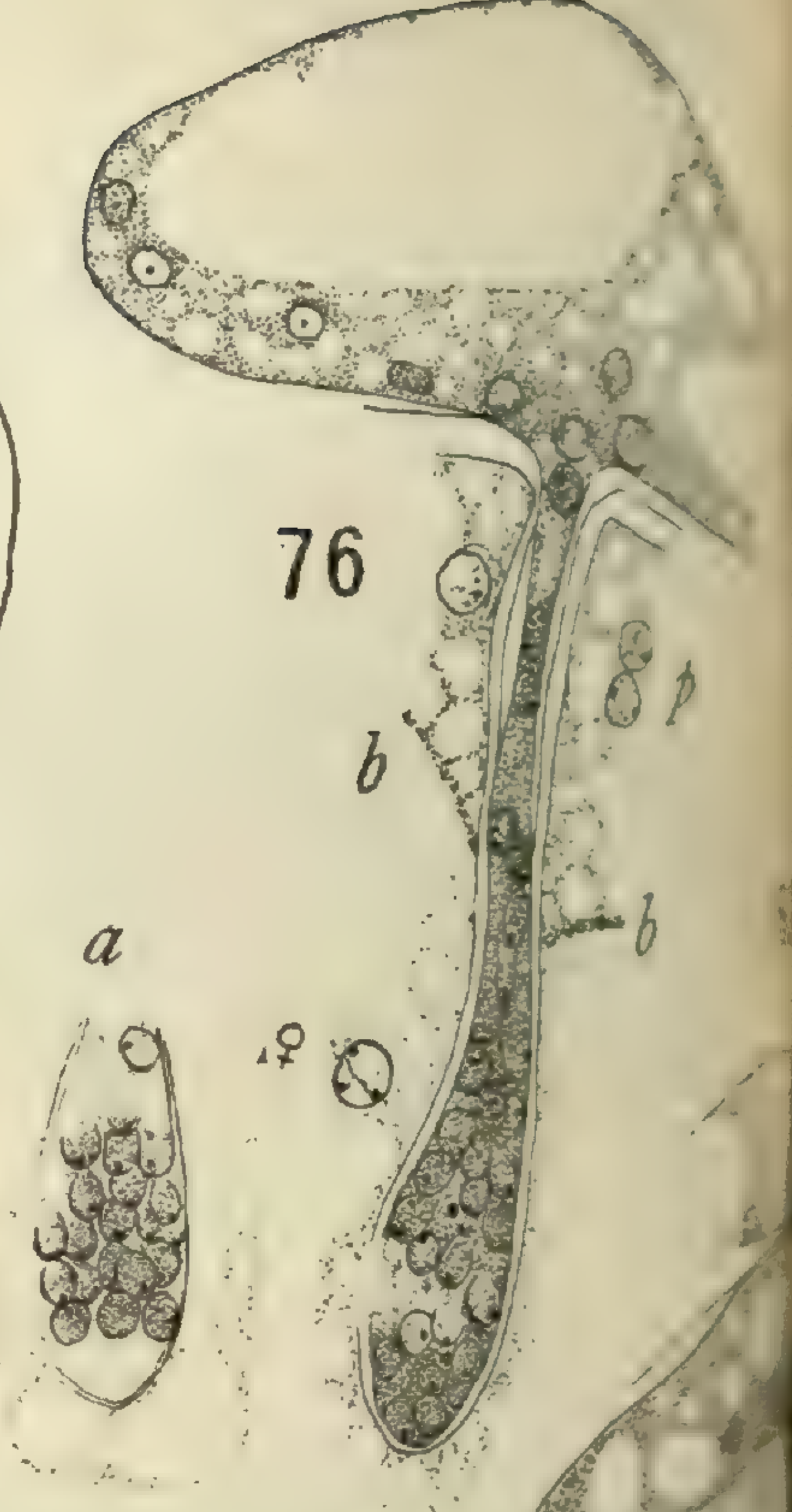
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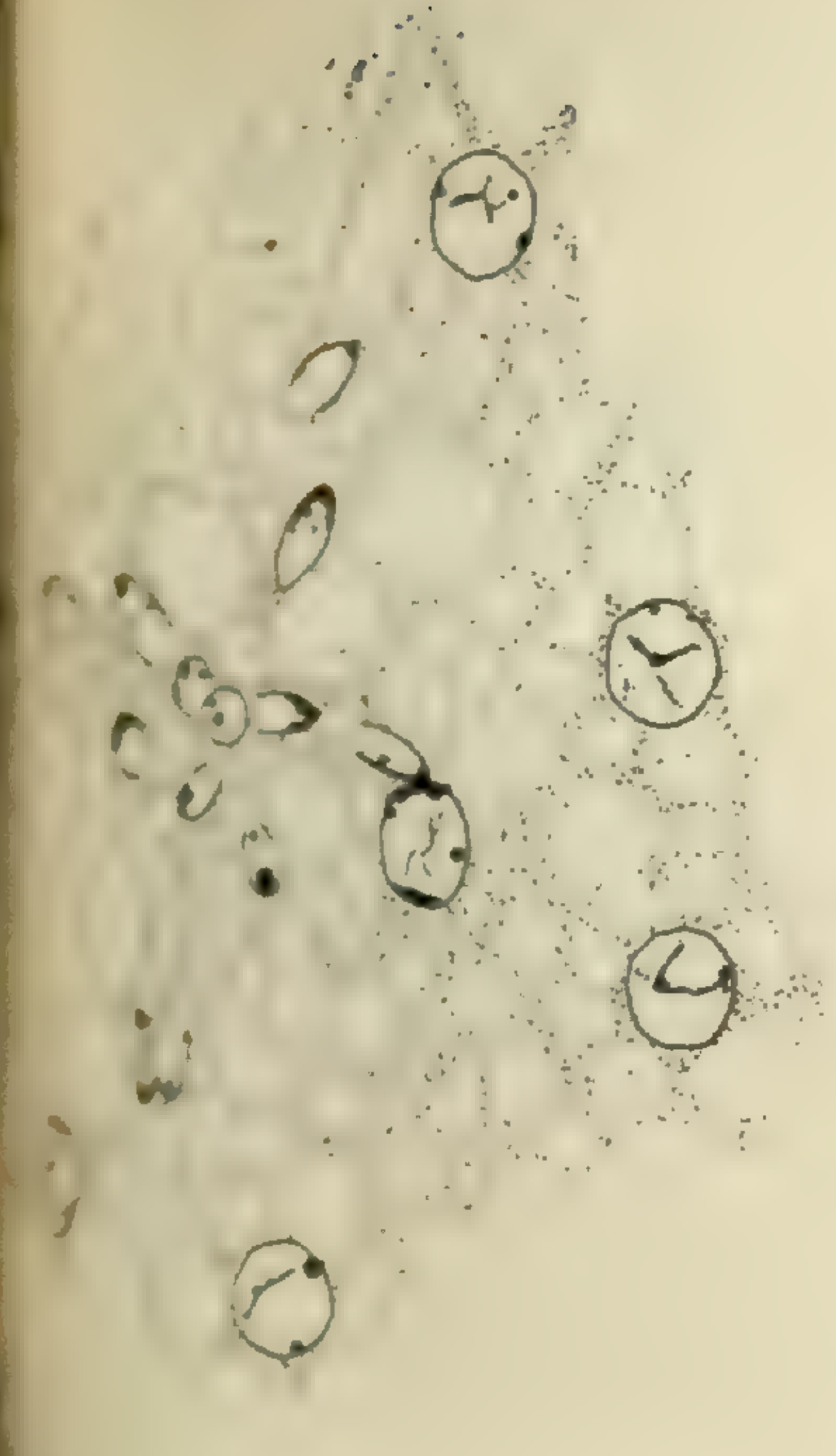

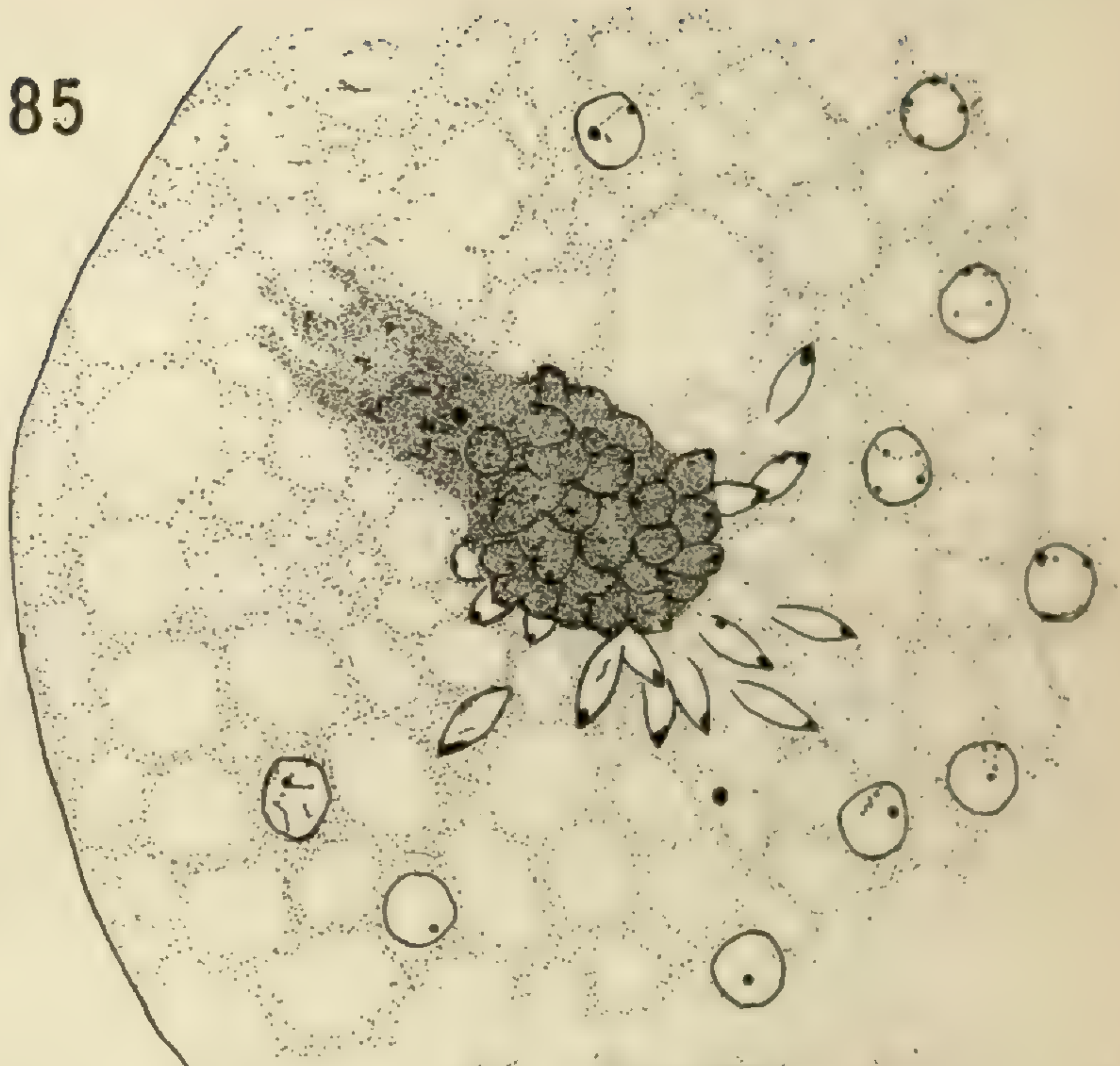
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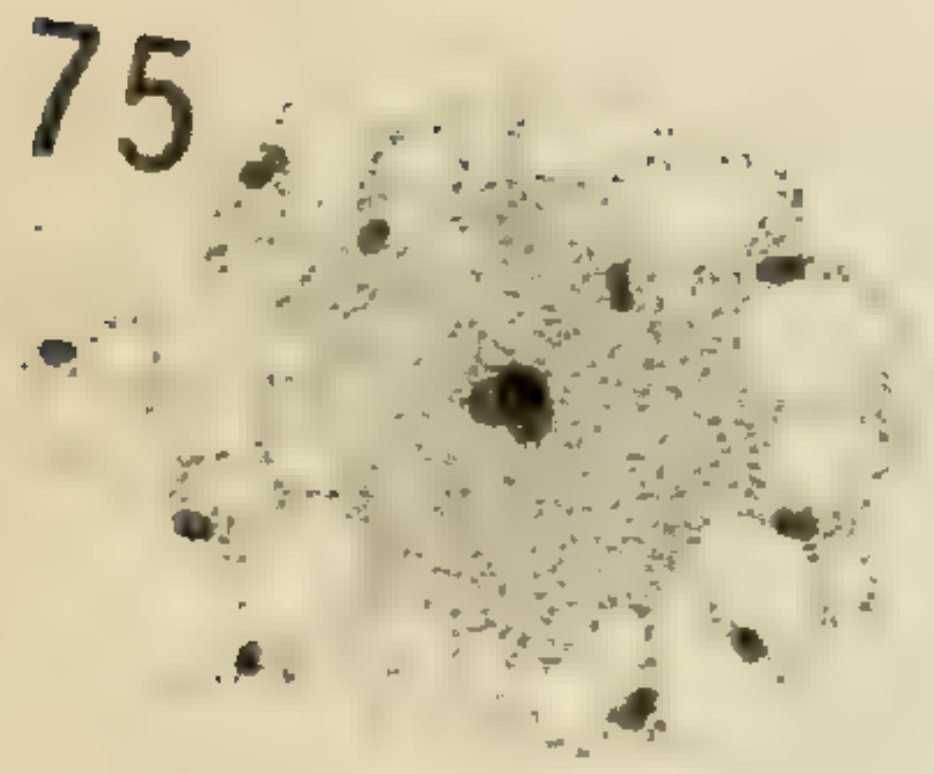
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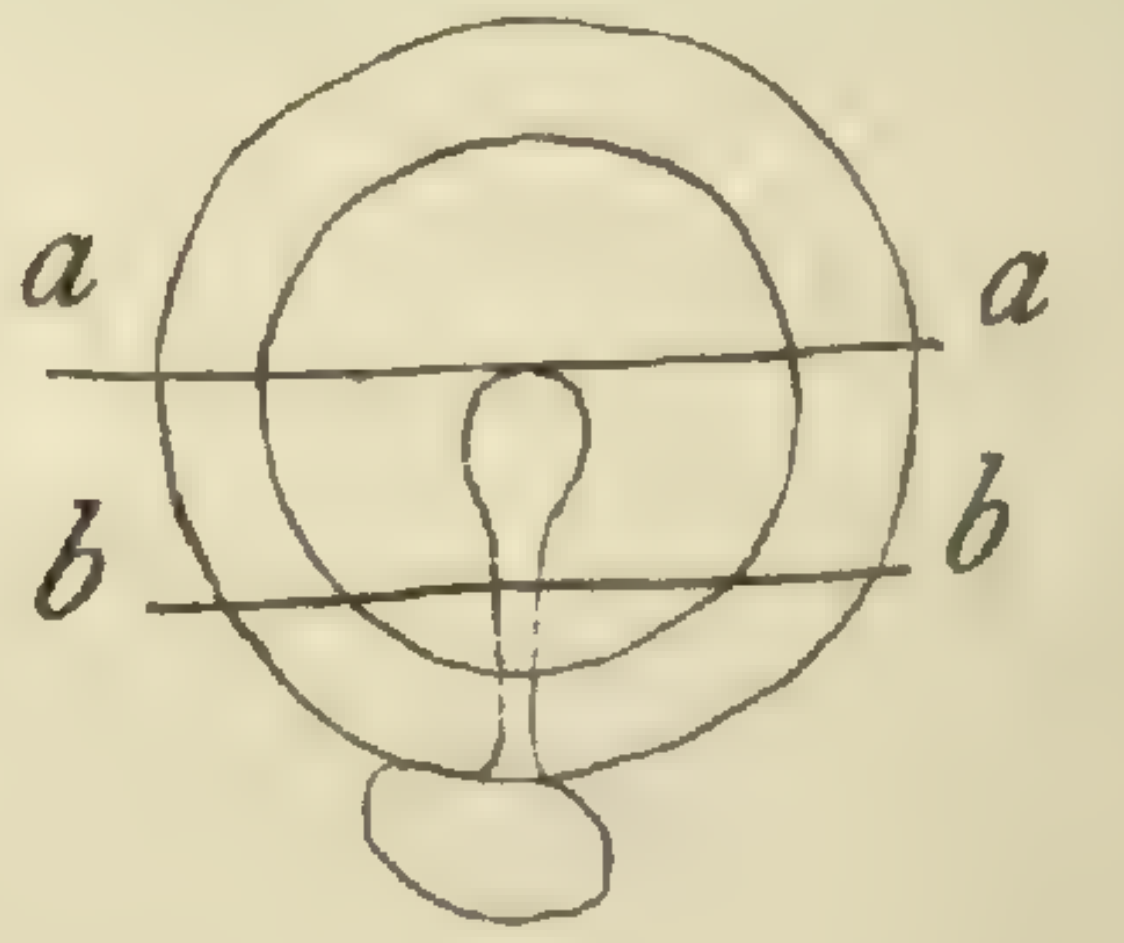
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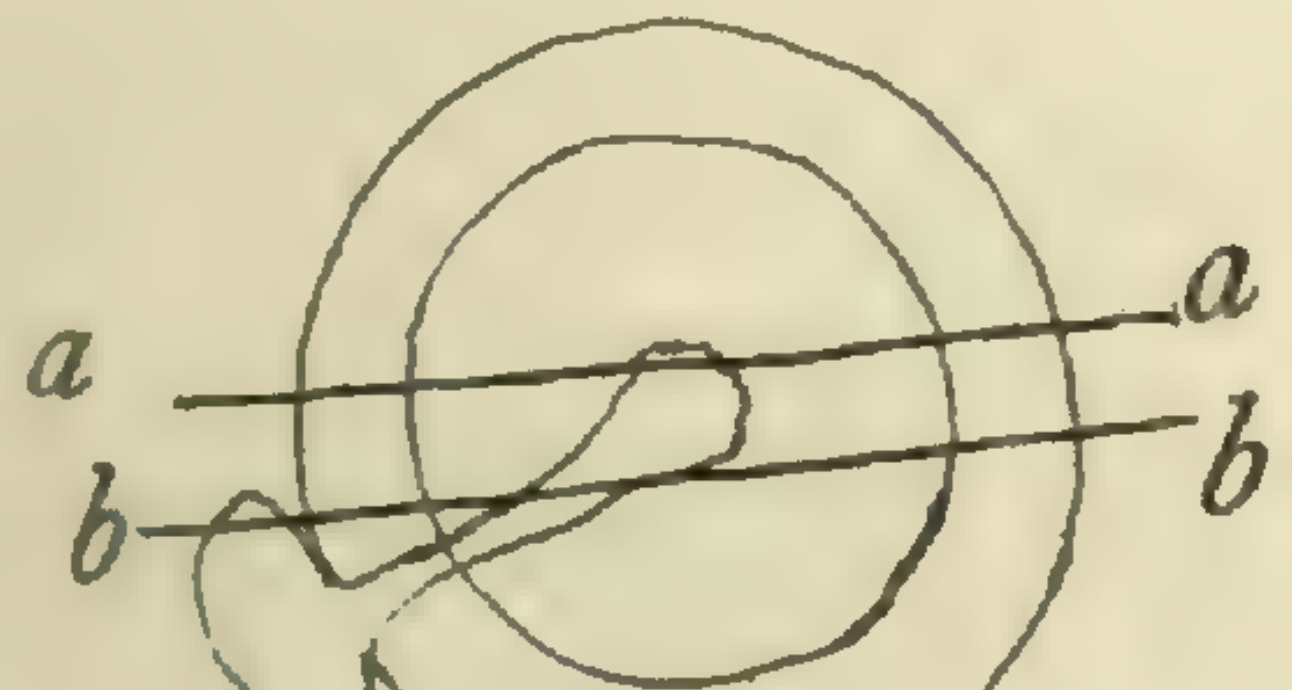
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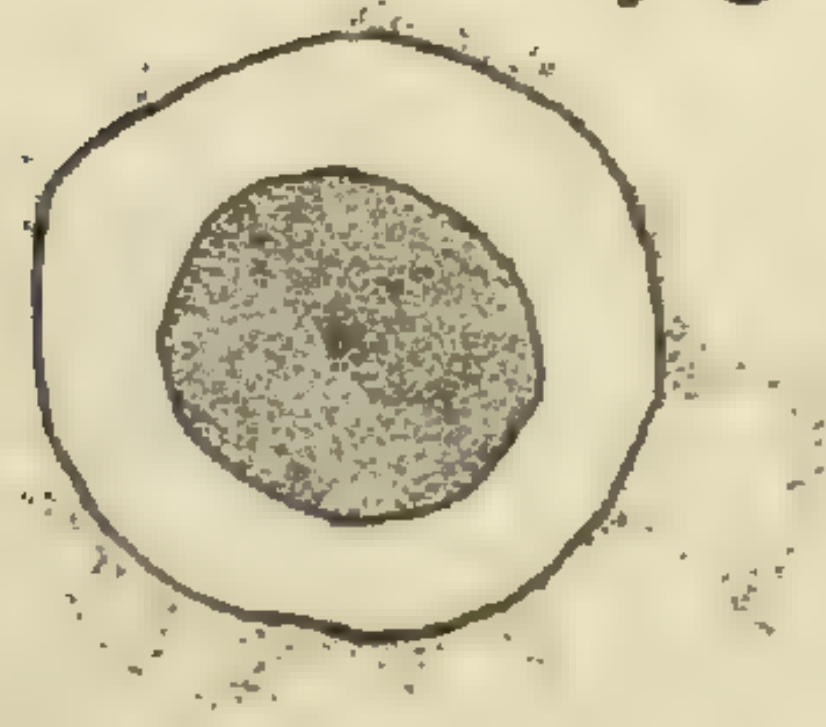
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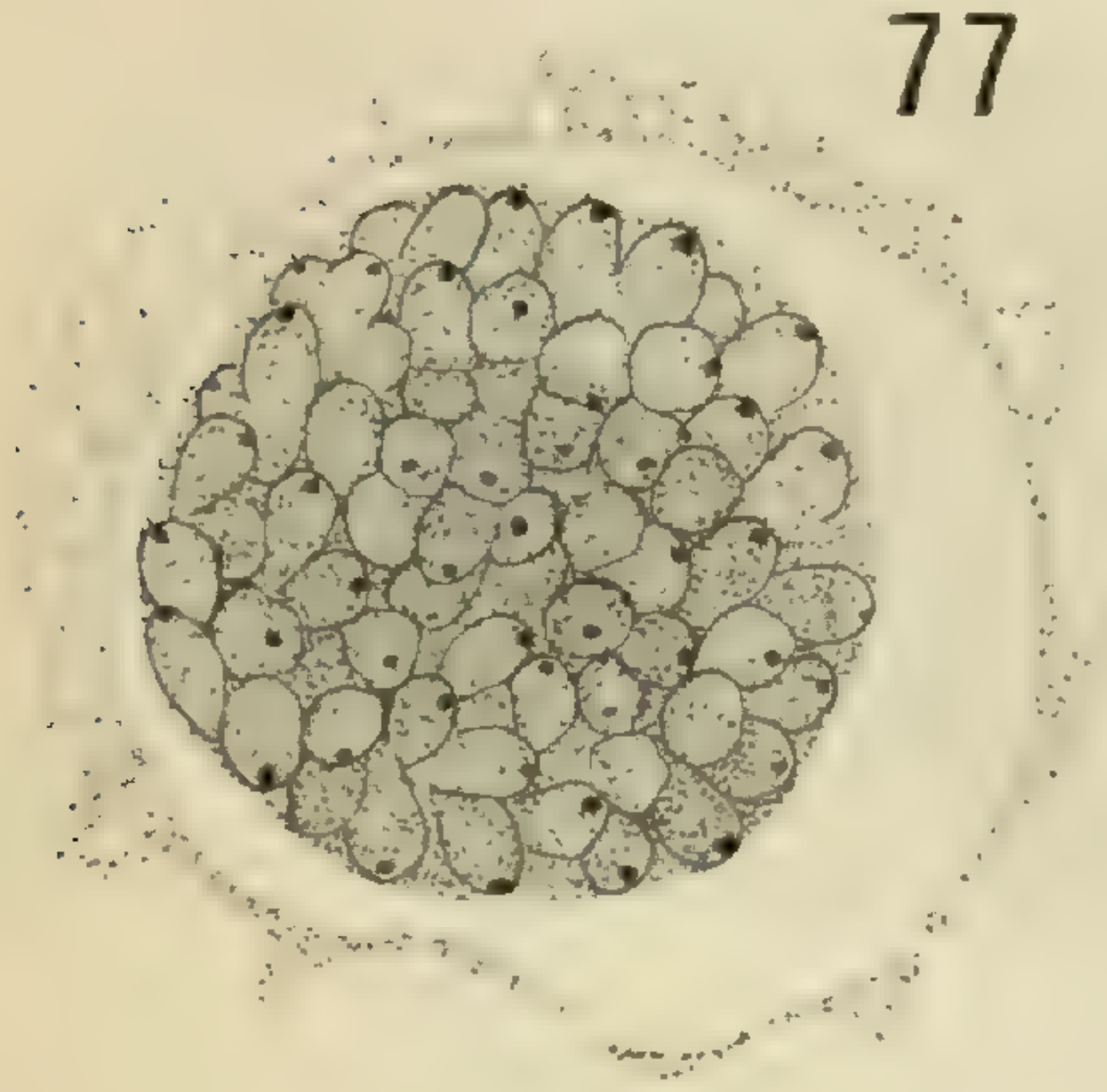
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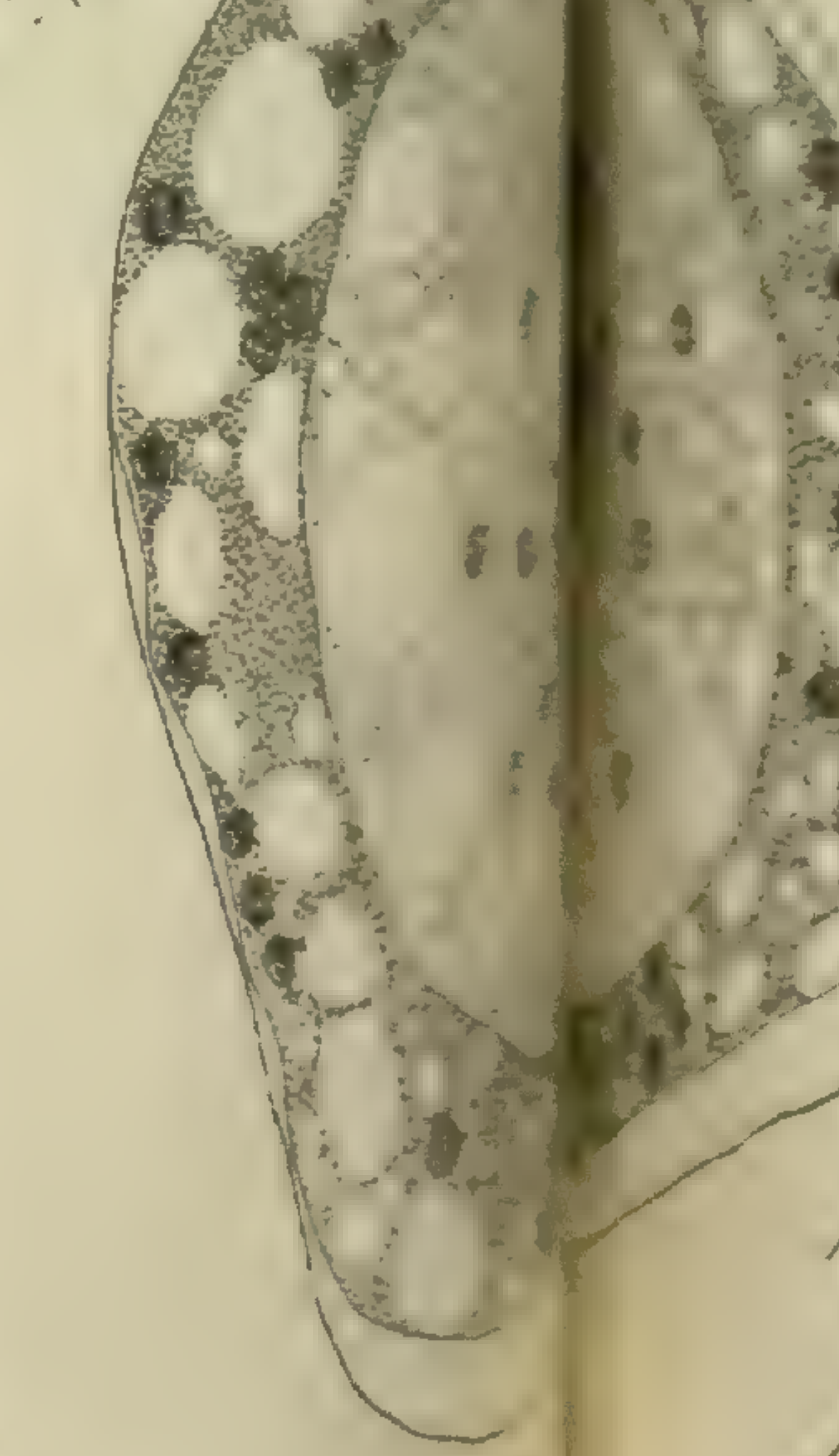

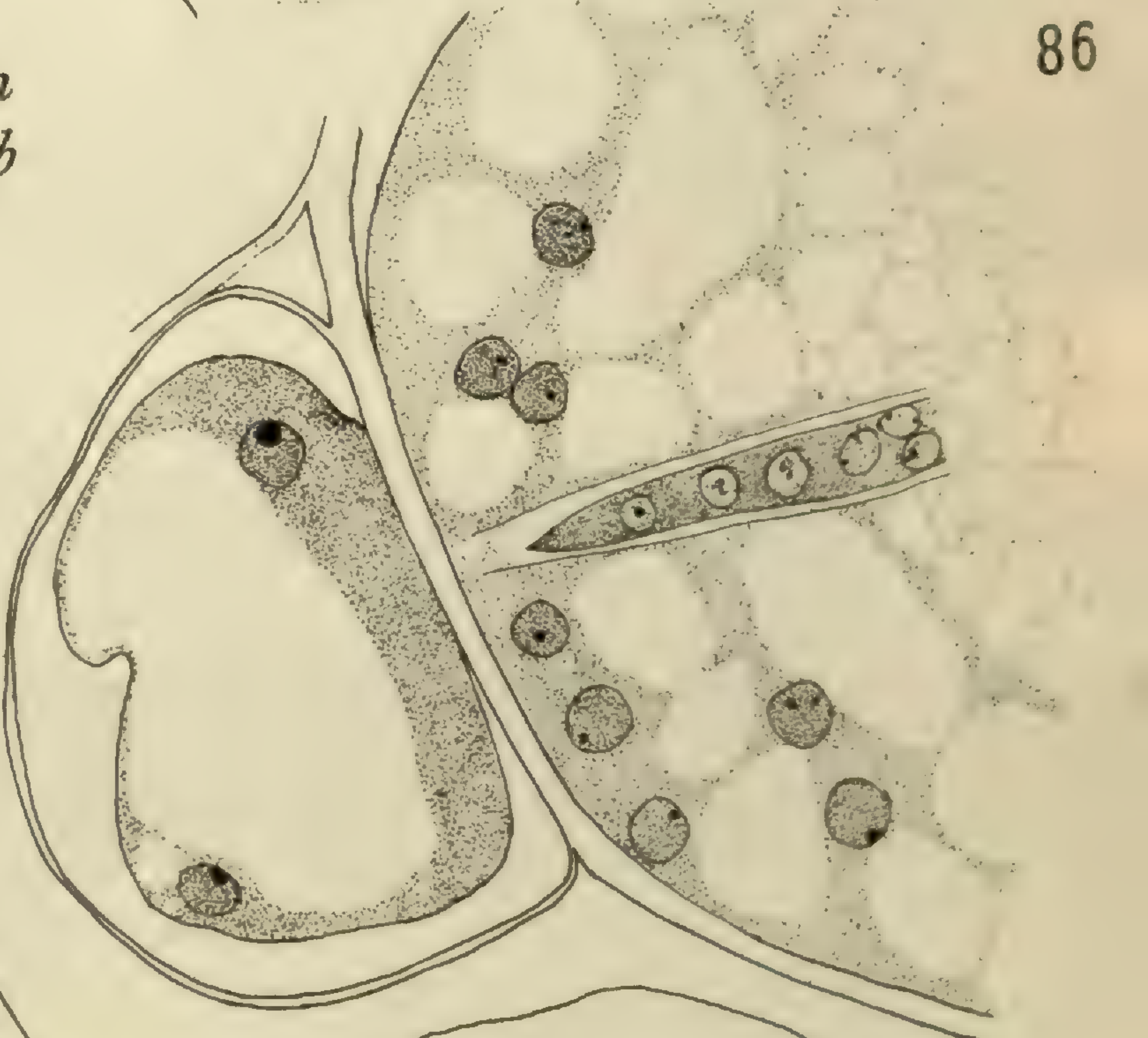
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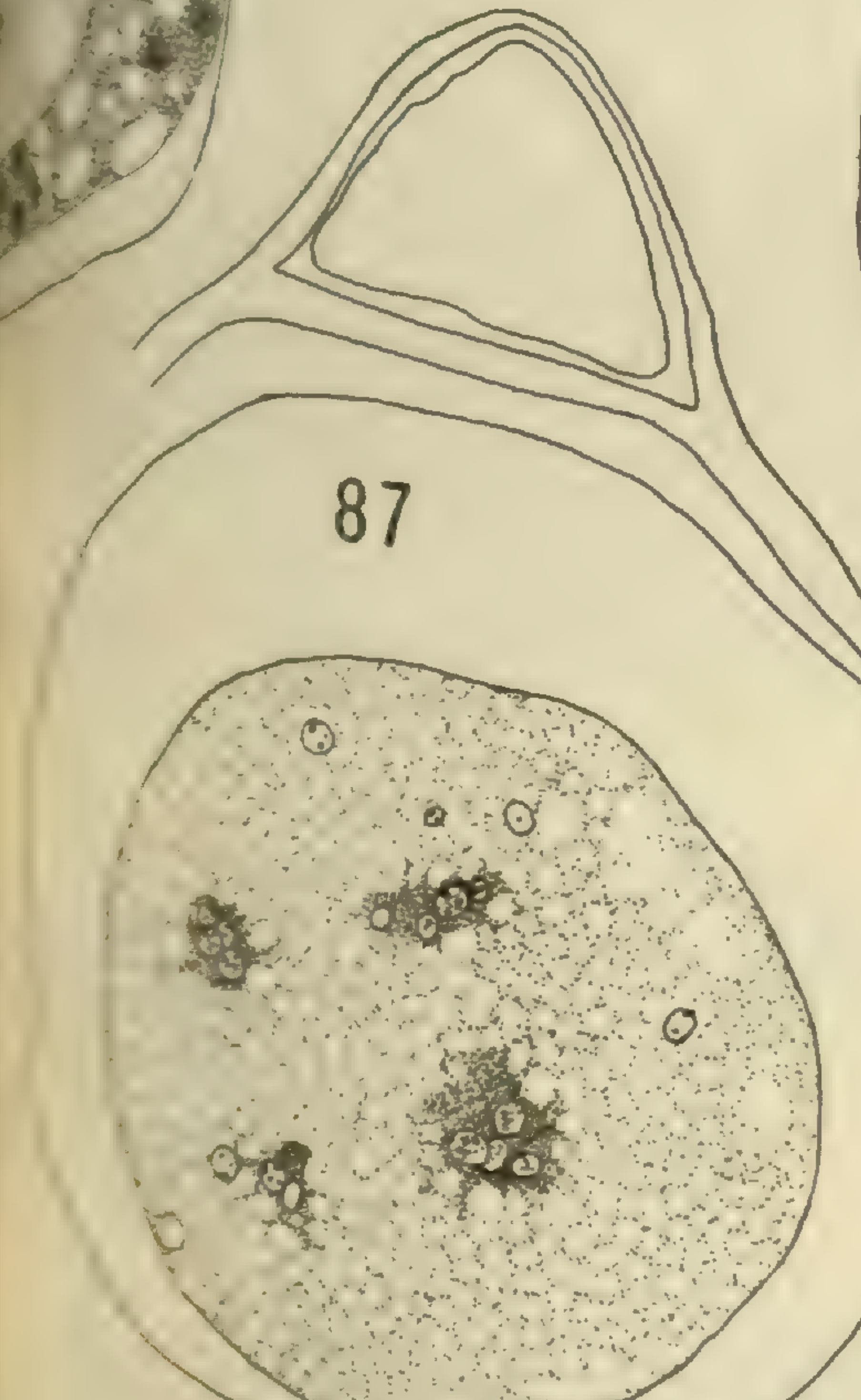
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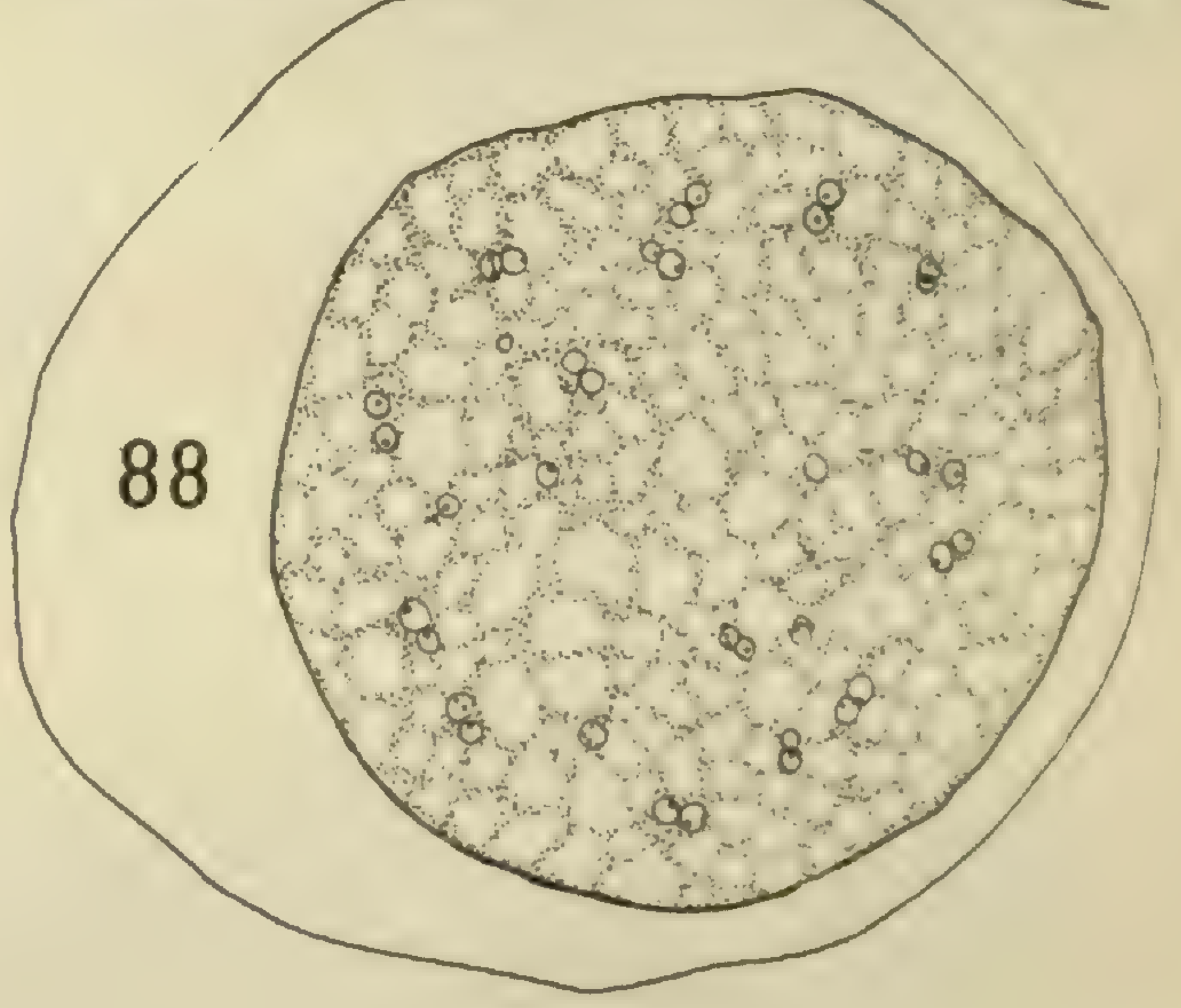
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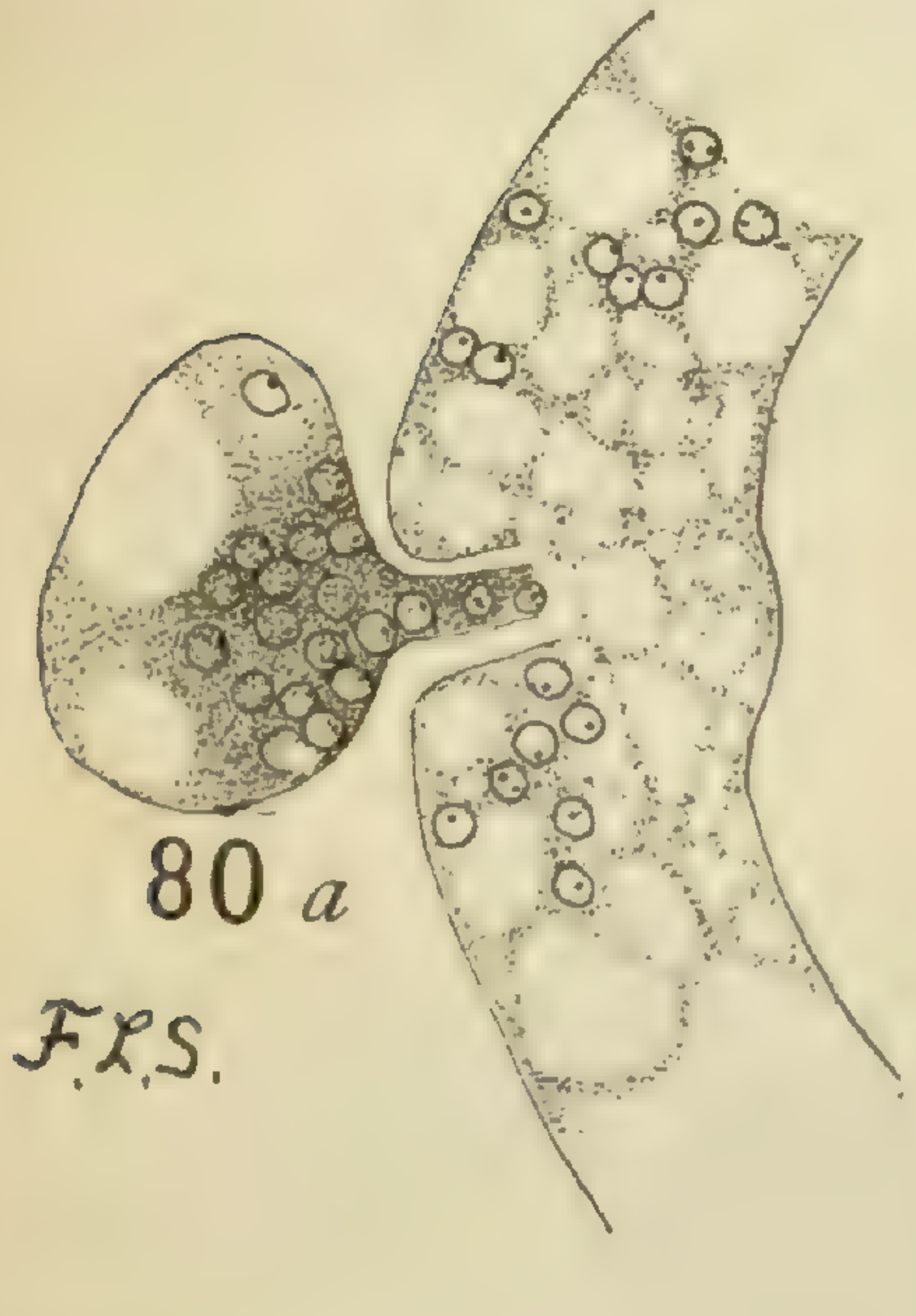
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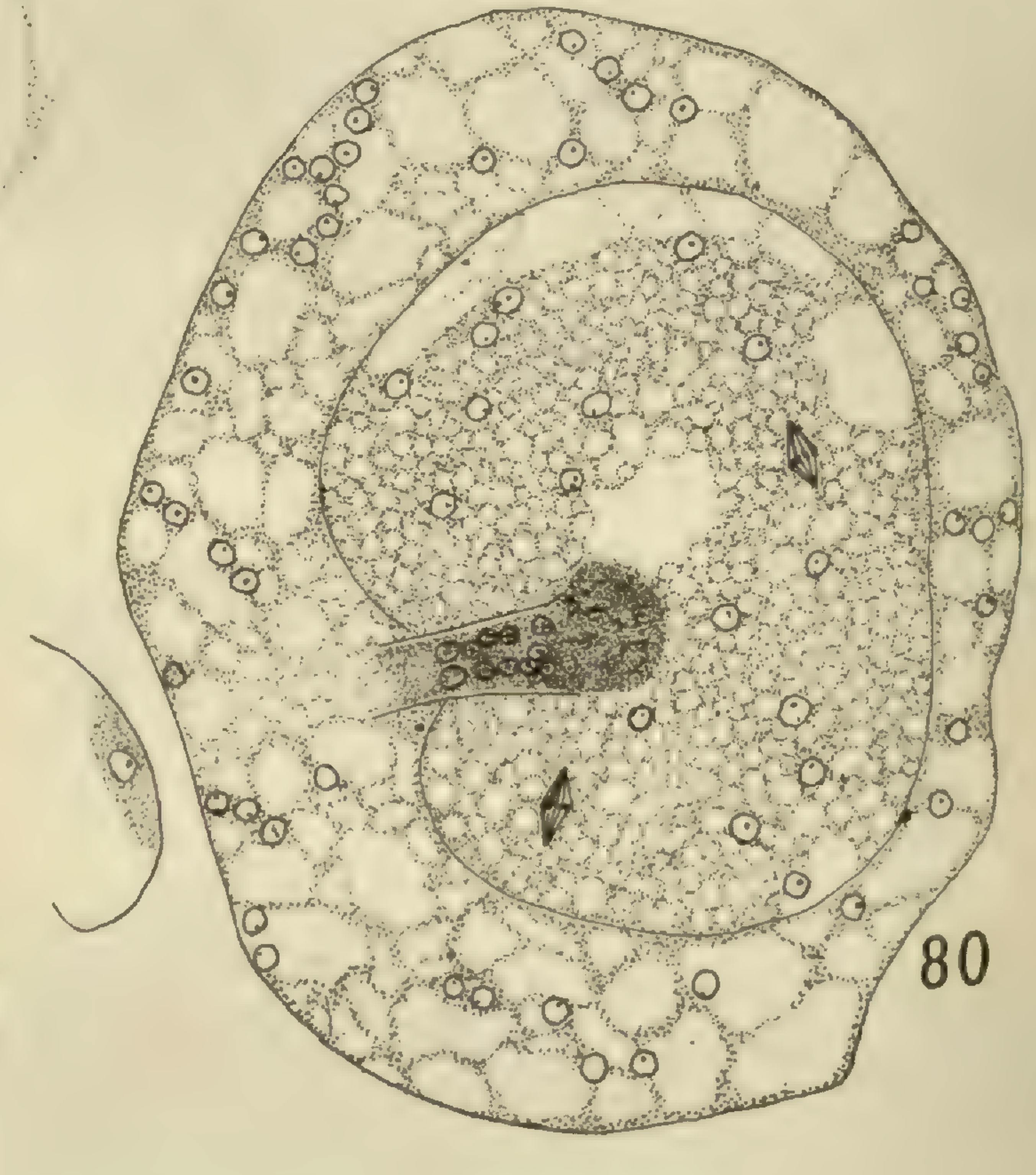
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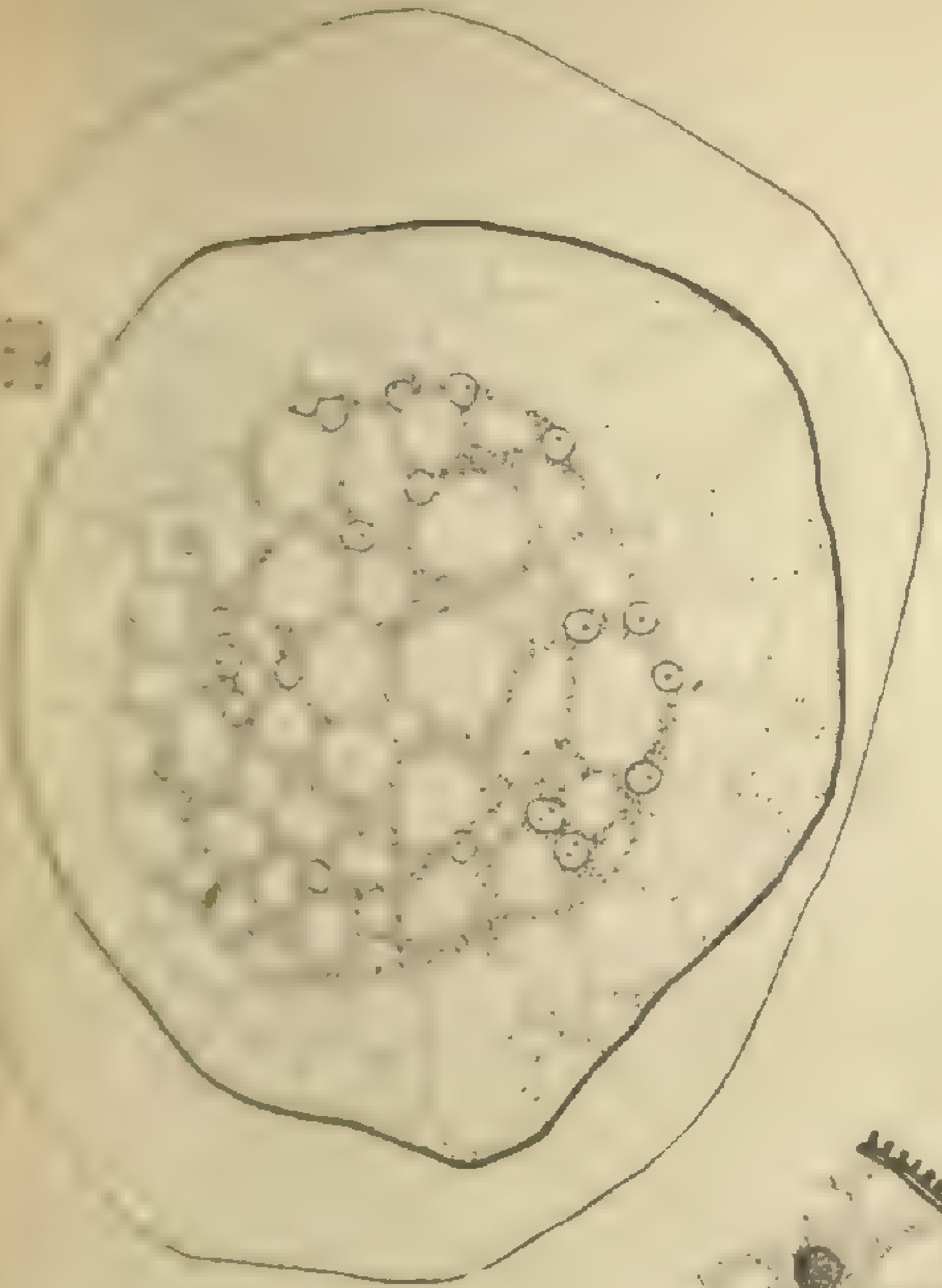

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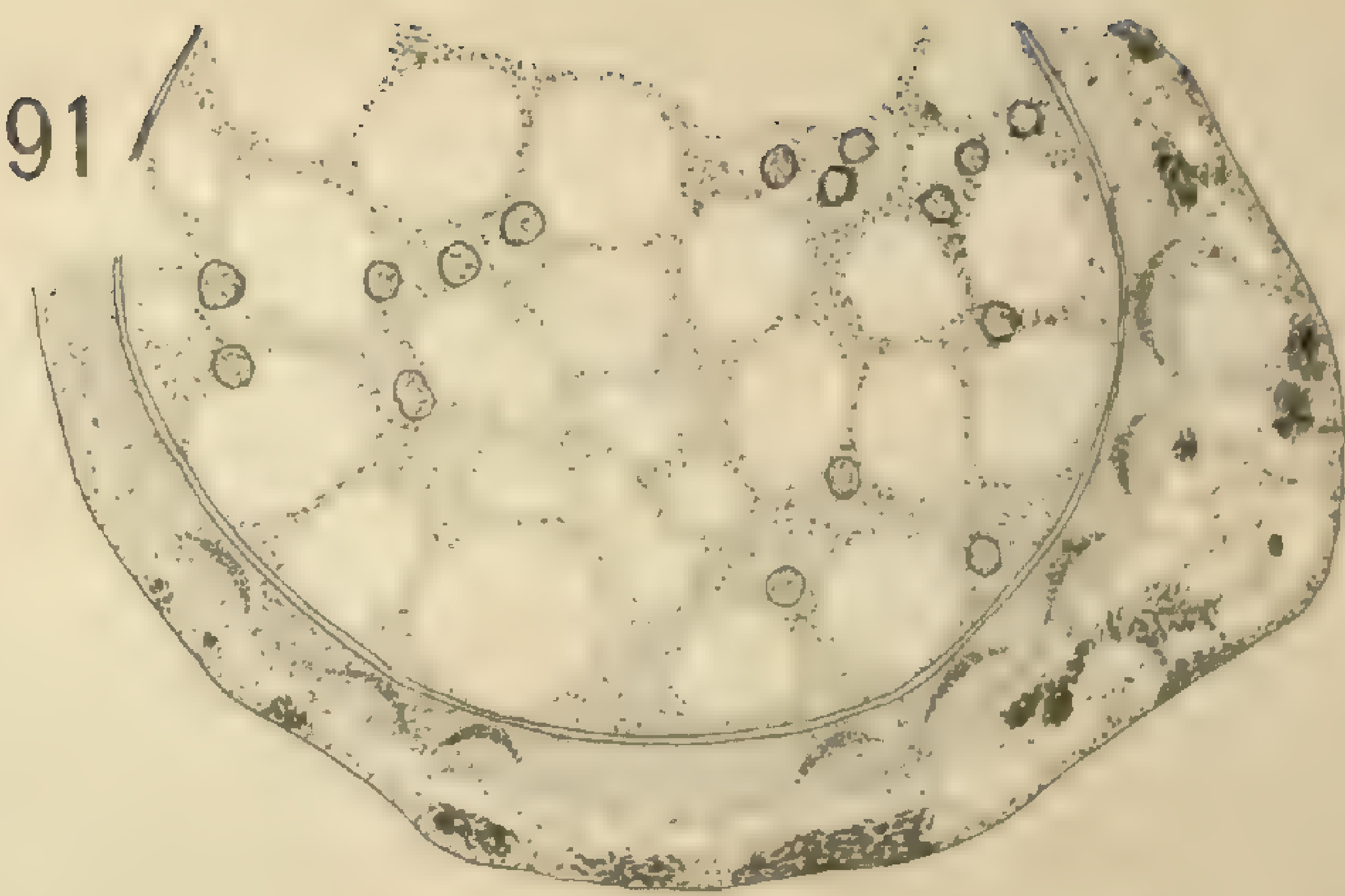

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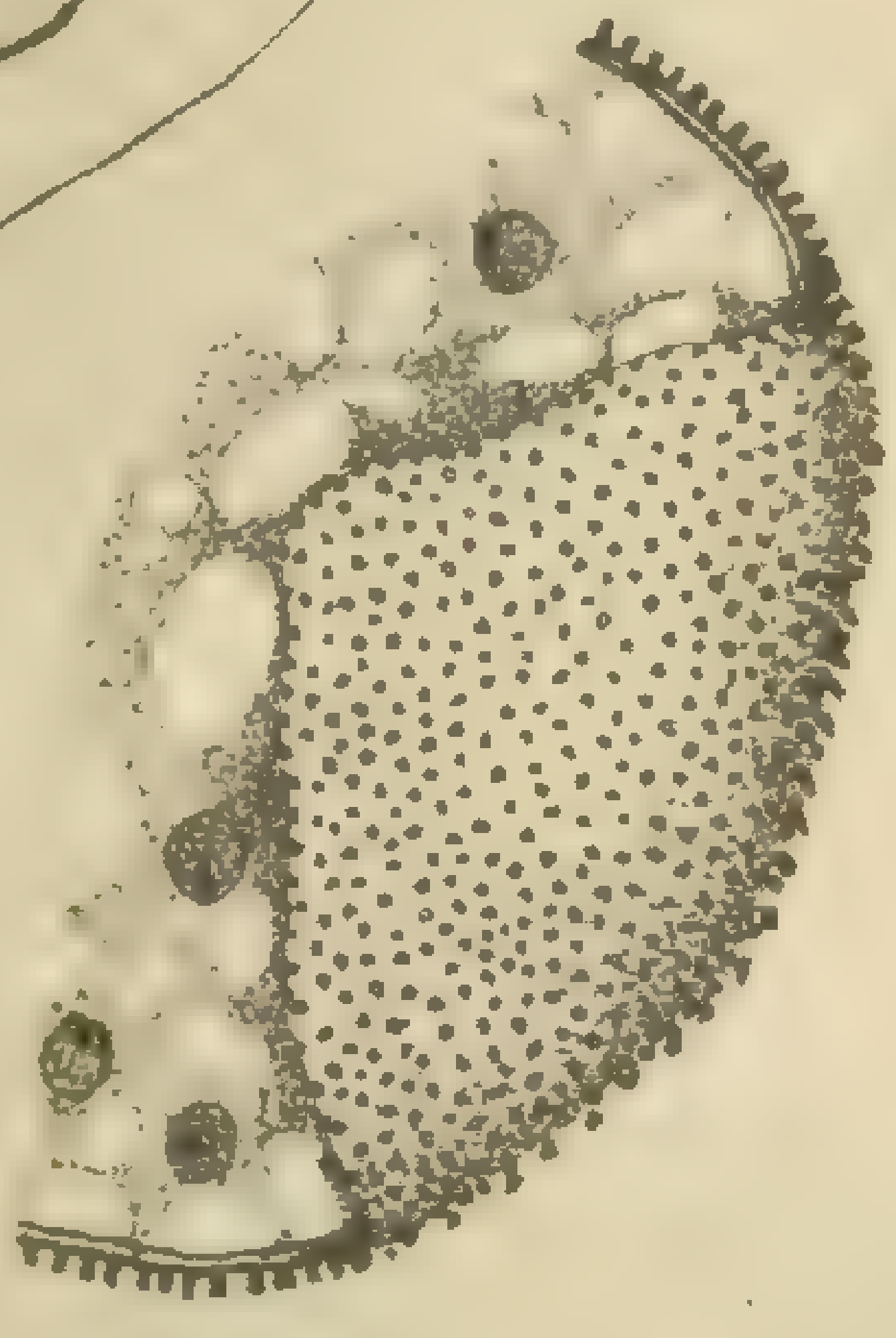

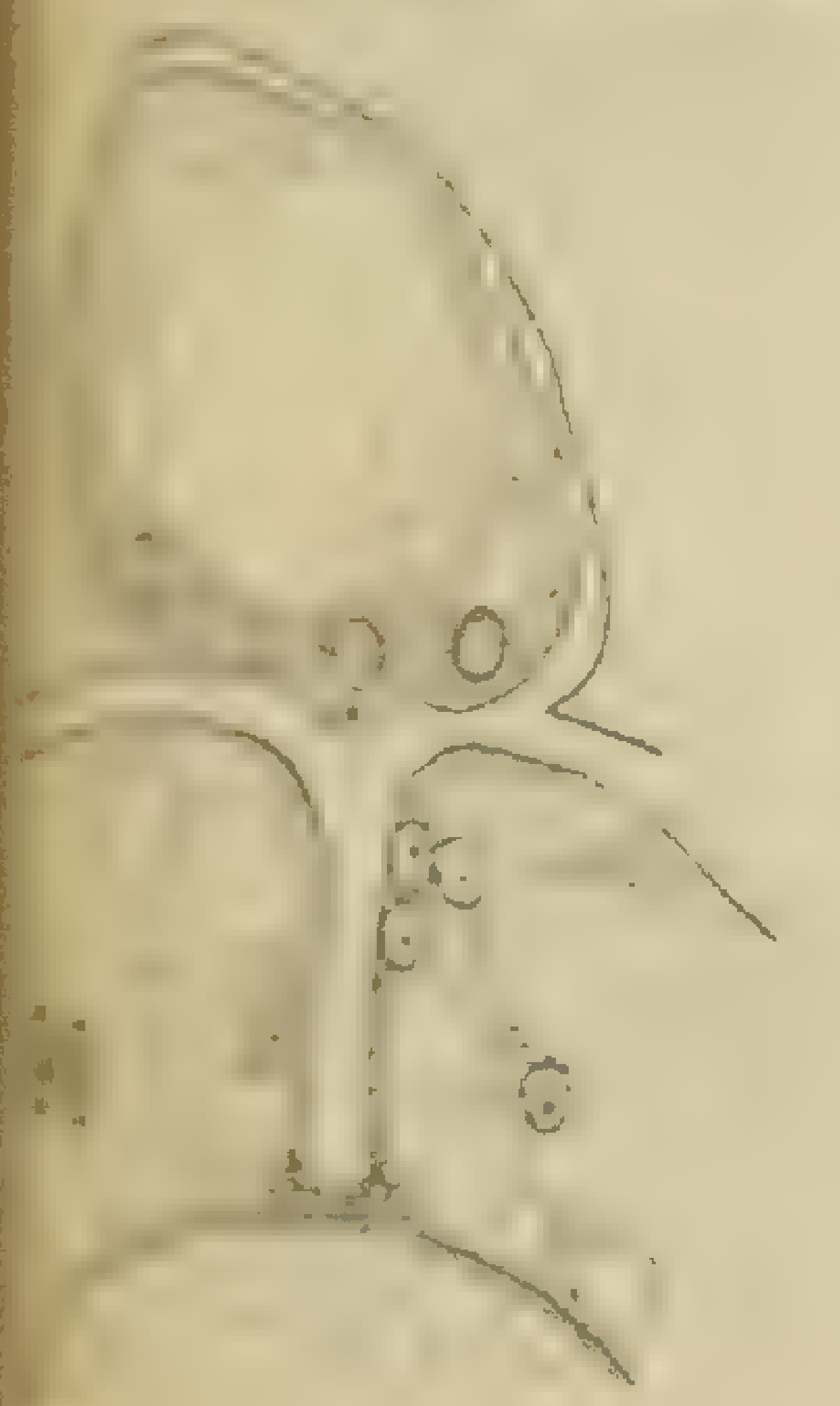

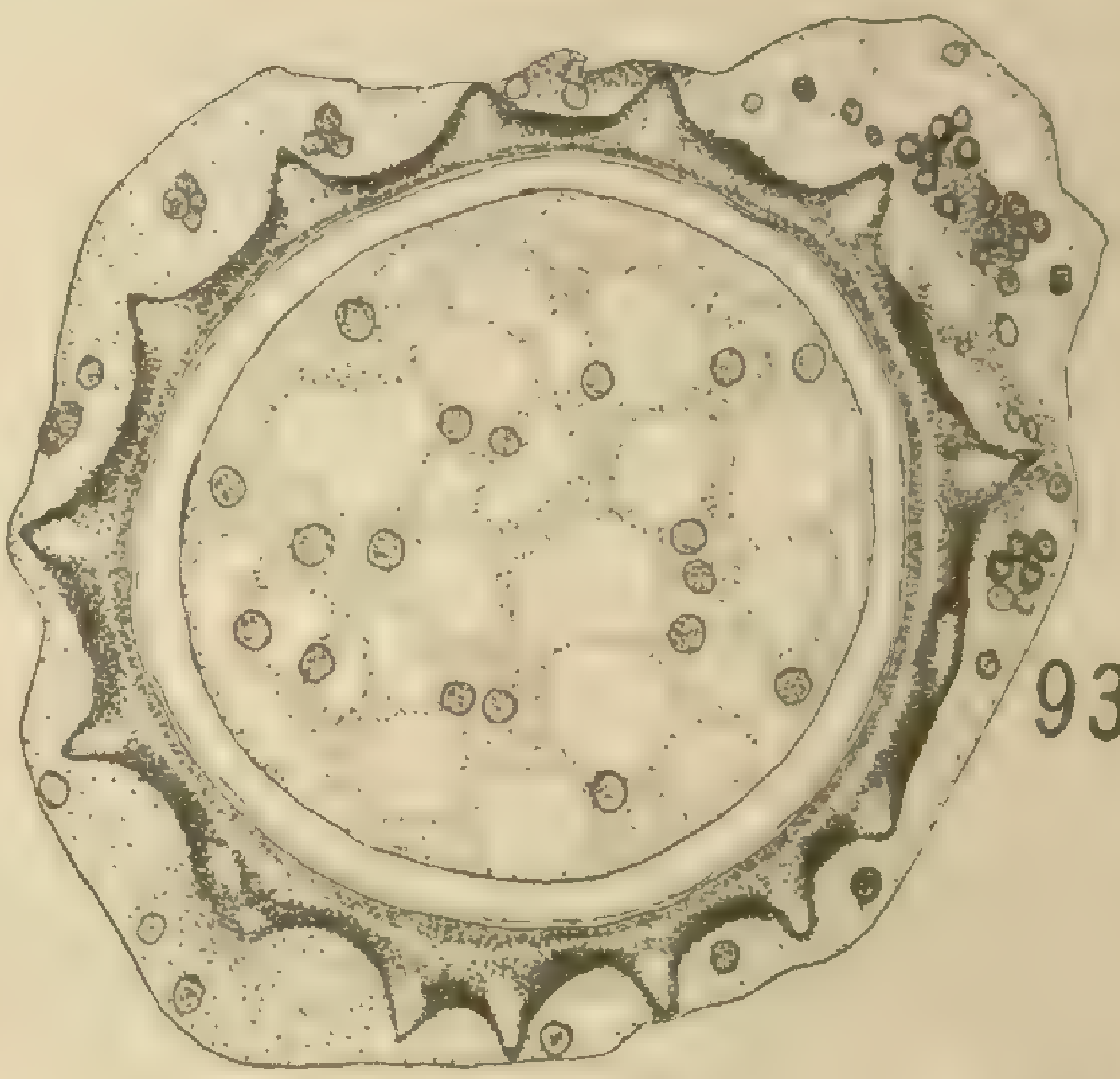

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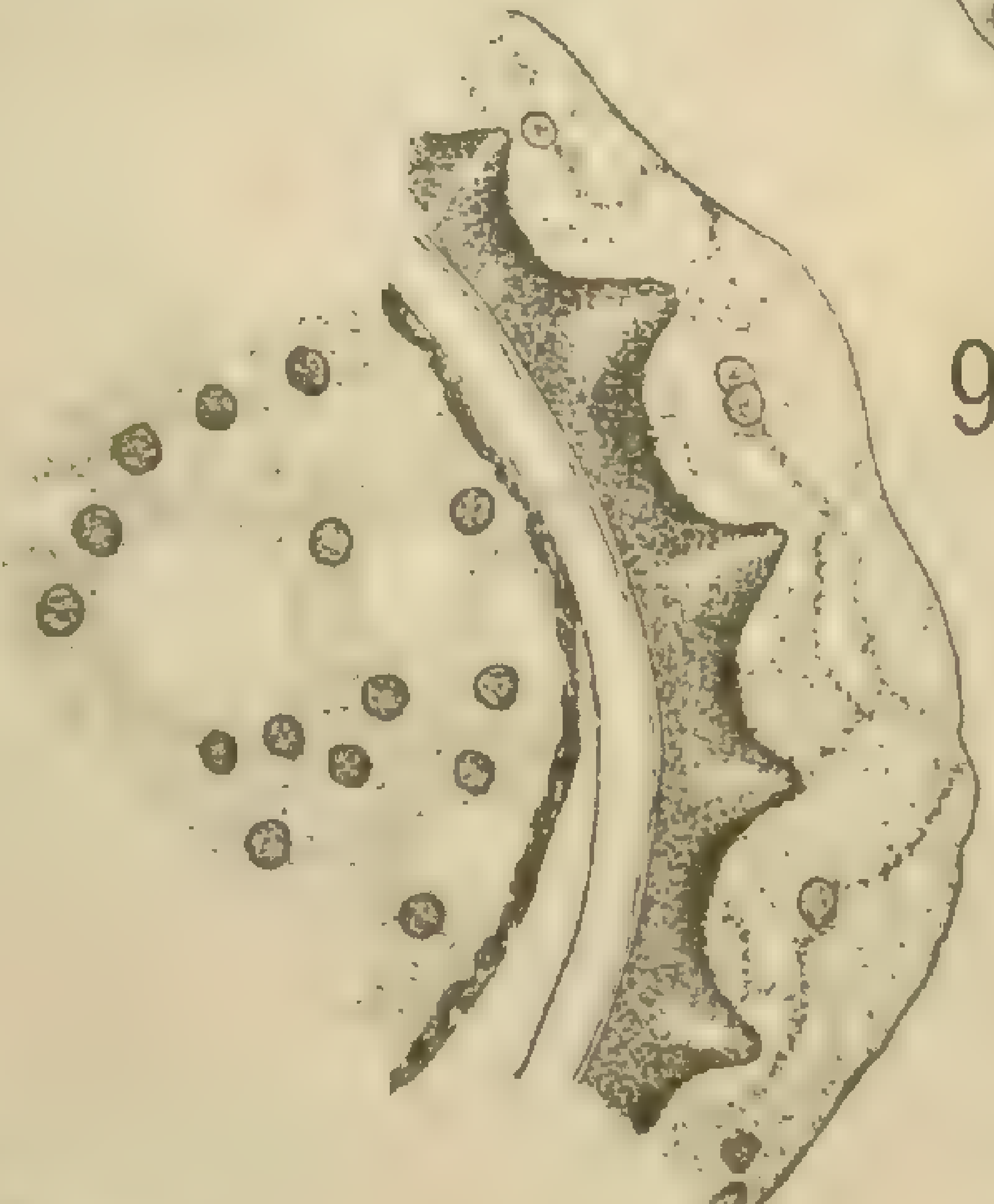

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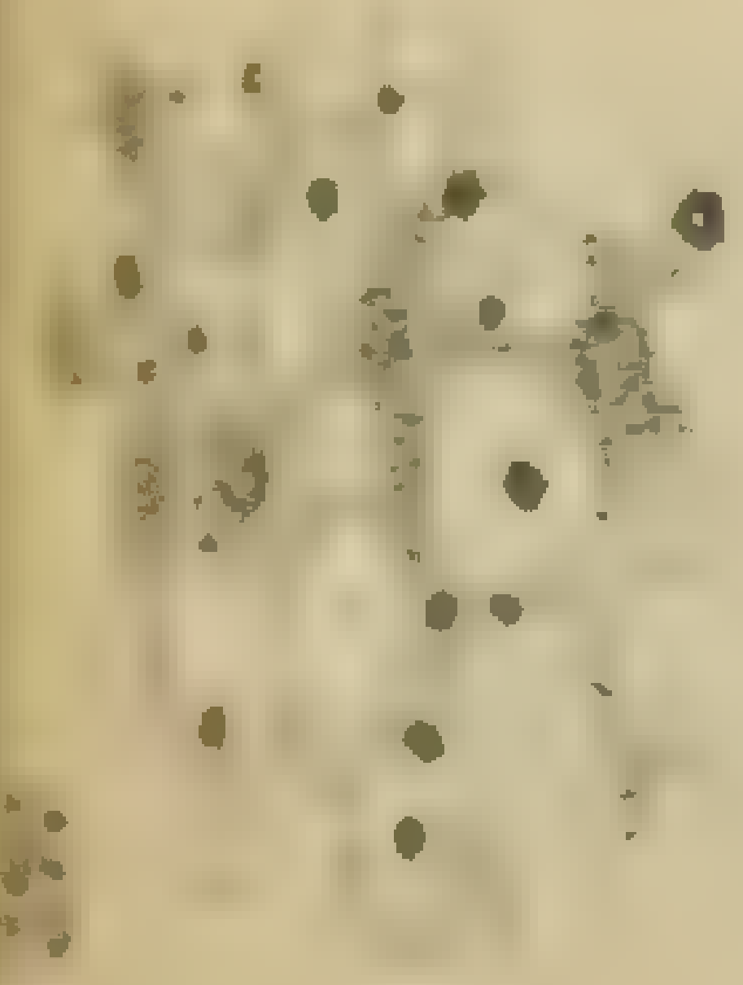

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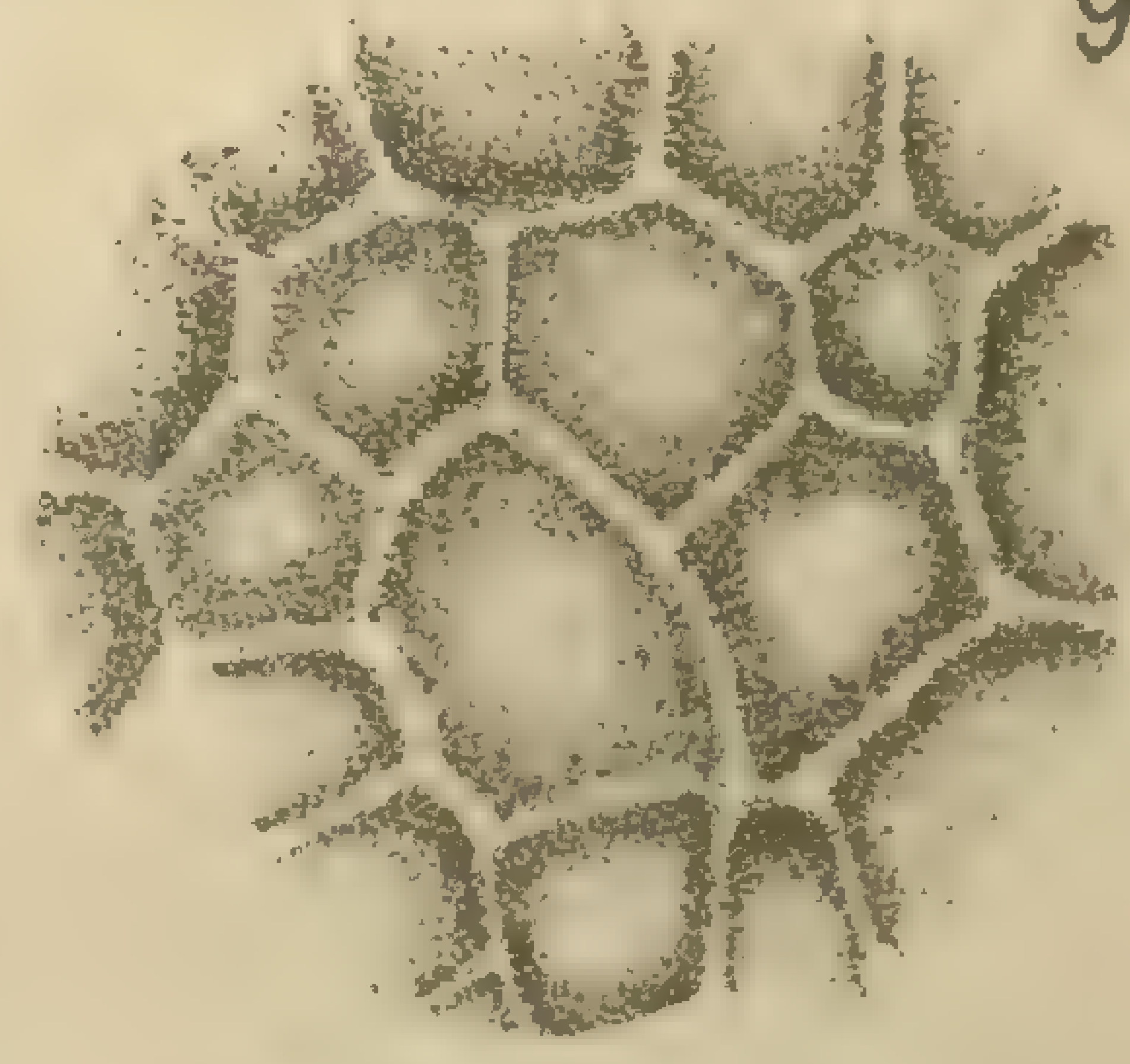

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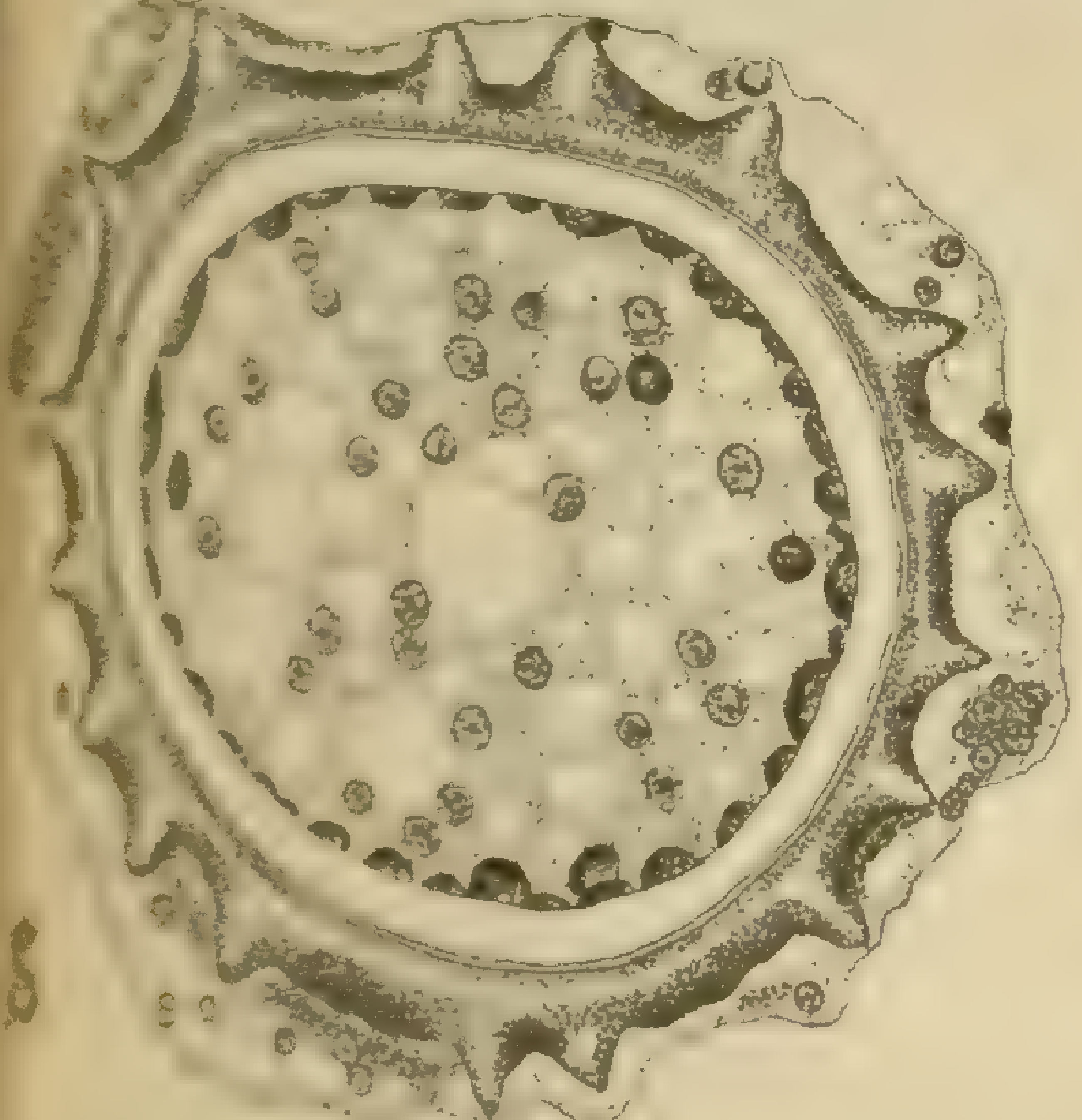

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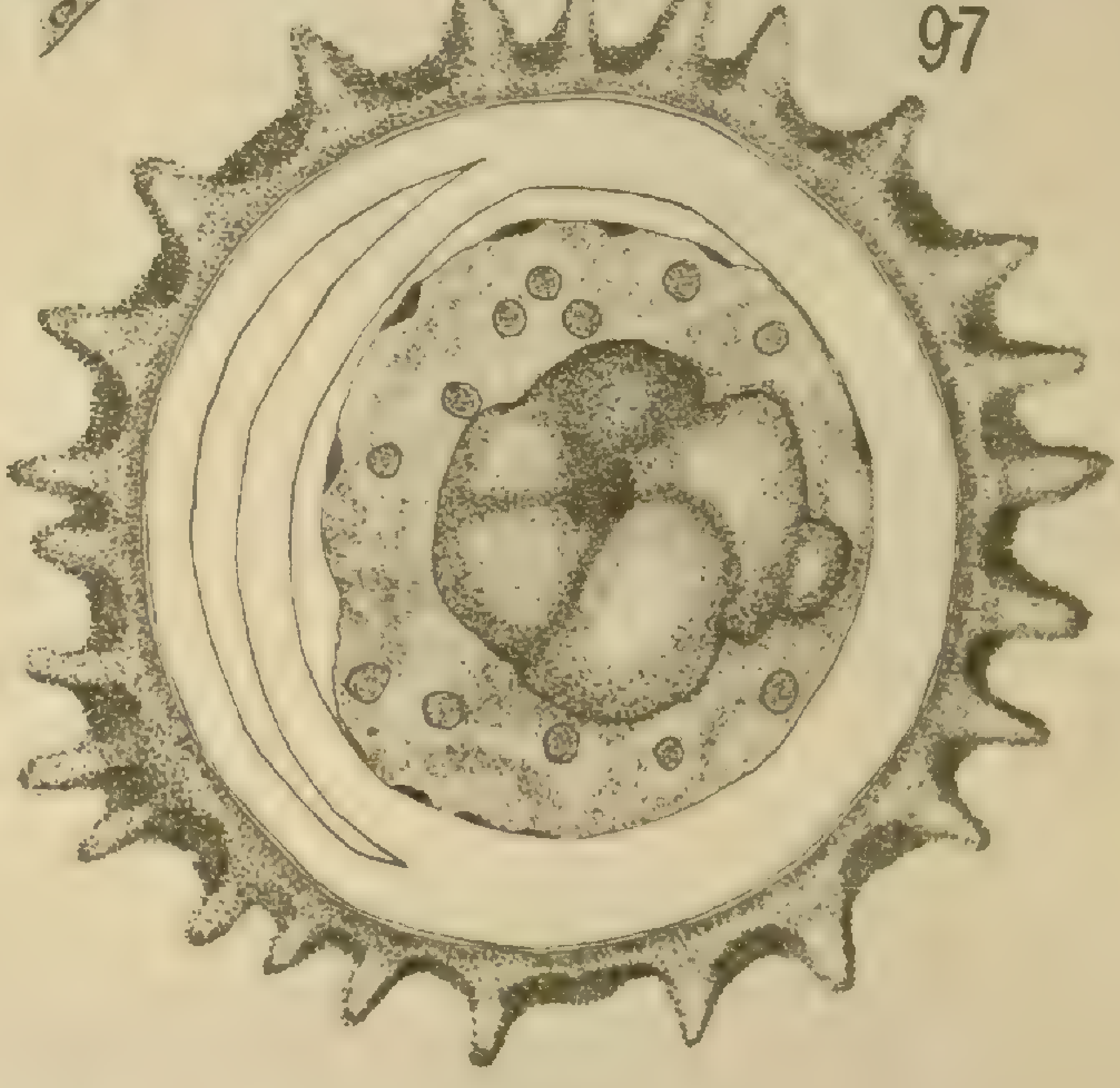

STEVENS on OOSPHERE OF ALBUGO

A BACTERIAL DISEASE OF THE SUGAR BEET.[1]

CLARA A. CUNNINGHAM.

(WITH PLATES XVI–XX)

In the autumn of 1890 Professor H. A. Huston, chemist of the Indiana Experiment Station, noticed that the analyses of some sugar beets showed a much lower per cent. of sugar than others, and the difference seemed to be associated with a slight change from the usual appearance of the tissues of the root. This observation led to a microscopical examination of the affected beets by Dr. J. C. Arthur, who discovered the presence of bacteria in the tissues, to which, after further study, was attributed their abnormal condition. During the year 1891–2 the characteristics of the disease were studied by Dr. Arthur and Miss Katherine E. Golden, and the results published in the form of a bulletin in 1892.[2]

This preliminary series of investigations determined that the disease was associated with a specific germ, which could be readily isolated from the diseased tissue.

No similar disease of the beet had been reported from any other locality in America at the time of the publication of this work. Dr. Ernst Kramer, in 1891, reported a bacterial disease of the beet root attacking the fodder beets of Russia, and almost simultaneously Dr. Paul Sorauer, of Germany, reported a disease of the sugar beet of that country. In the *Export* of 1894,[3] Dr. Sorauer gives his opinion that the disease of the fodder beet, named by Kramer "bacteriosis gummosis," and that of the sugar beet similarly named by himself "bacteriose gummosis," are identical, and, perhaps, very closely related to the bacter disease of sugar cane known as "sereh."

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[1] Read before the Society for the Promotion of Agricultural Science at the Boston meeting, August 1898.

[2] Diseases of the sugar-beet root. Purdue University Agric. Exper. Station, Bulletin no. 39.

[3] Export, 1894, no. 30.

1899]

The diseased beets, as observed in Russia, are described having dried leaves with withered heart leaves. The roots badly diseased beets were so tough they could scarcely broken, the broken surfaces soon turning black. These be produced a pathogenic effect on cattle to which they were Many of the diseased beets, when first sectioned, appeared fectly sound, but after a few minutes the fibrovascular bun turned dark and a syrup-like gum exuded from the cells. other beets the tissue was sometimes completely broken d

Dr. Sorauer says: "The similarity between the beet and sugar-cane disease 'sereh' consists in the destruction of the c sugar and the increase of the invert sugar as well as in the co ing of the vascular bundles and the entrance of bacteria." also believes that the disease discovered in America by Ar and Golden may be the same as that determined by Kramer a himself in Europe.

Mr. Walter Busse, in 1895, took up anew the study of the *bacteriosis gummosis* of the sugar beet, the material for s being sent him by Dr. Sorauer. In describing the diseased he speaks of the gum-like fluid as follows: "Soon after t drops appear on the surface of the sectioned beet they covered by a thin black membrane, which consists of sm black, round bodies of different sizes."

The aim of Mr. Busse was, first, to determine the form bacterium common to all the diseased beets by the separation the germ from the diseased tissue; and, second, to demonst that this germ was the specific cause of the disease by inocul healthy beets with the germ. In the first series of experime three germs were isolated. Two of these were discarded the third form was kept for further observation. This f appeared as short rods 1.72–2 μ long and 0.8 μ–0.9 μ br They were actively motile, and grew well in cane or grape s solutions, producing an abundance of gas. This form was but from other diseased beets a second gas-producing form isolated, resembling the first in form and arrangement, but smaller, being 1.5 μ–1.75 μ long and 0.7–0.8 μ broad. This form produced an acid reaction on different media, did not liquefy gelatin, and grew better at 12°–14° than at 22°. Mr. Busse is inclined to believe that the second form is a variety of the first, which he designates as *Bacillus Betæ*. He has demonstrated that this second form produces the disease known as *bacteriosis gummosis*, and believes that this germ is a saprophyte which becomes a parasite in the tissues of the beet.

Erwin F. Smith,[4] in speaking of the bacterial diseases of the sugar beet as reported from Europe and America, is of the opinion that the diseased condition of the beets studied by Arthur and Golden is due to some other cause than a bacterial one. He states that it is highly improbable that the root could be attacked by an organism which invades its tissues and yet does not break them down. Mention is made of the fact of the presence of small bodies in the tissue of healthy beet roots which have the appearence of bacteria, but which are probably crystalloid bodies. A paper by Dr. Smith was presented at the meeting of the Society for Plant Morphology and Physiology in December 1897, calling attention to the "existence, in parts of the United States, of a disease of the sugar beet resembling if not identical with that described by Kramer and Sorauer in 1891–2, and more recently by Busse."

In the fall of 1896 I had the opportunity to continue the investigation of the bacterial disease of the sugar beet observed in Indiana in 1890–1. Much of the value of my experimental study of this disease is due to the suggestions of Dr. Arthur, to whom I am indebted for kindly help and criticism of my work. I also desire to express my gratitude to Professor Burrage, Professor Huston, Miss Golden, and Mr. H. L. Bryan, also of Purdue University, for important suggestions. My investigations, which have been continued from 1896 to the present time, have resulted in no positive evidence that the sugar-beet disease of Indiana is the same as that described by Sorauer and Busse of Europe. The points of similarity will be noted in the following description of the disease and of the germ by which it is produced.

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[4] SMITH E. F.: Am. Nat. 30:716–729. Sept. 1896.

GENERAL DESCRIPTION OF THE DISEASE.

About the middle of September 1896 several diseased beets were found in the field of cultivated beets on the grounds of the Purdue Experiment Station. The disease attacks the whole beet plant, causing a peculiar appearance of the leaves, so that with a little practice the diseased beets can be distinguished readily from the healthy ones as they grow in the field. The outer, older leaves soon die away, and the intermediate and heart leaves are left wrinkled, curled, rather flabby than turgescent, and of a yellowish-green color. This wrinkled appearance is caused by blister-like patches being formed between the veins of the leaf, and the whole has been described as resembling a Savoy cabbage leaf. See photographs of leaves, *plate XVI*, and also photographs of diseased beest, *plates XVII*, and *XVIII*.

The appearance of the exterior of a beet root when diseased is not materially different from that of the healthy beet. It is perhaps not quite as brittle. A decisive test for the disease is found in the appearance that the root shows when sectioned. The fibrovascular bundles appear as dark rings in the white flesh. They grow almost black after being exposed to the air for a few minutes. These rings are quite distinct from the cream colored fibrovascular bundles of healthy beets (*plate XIX*).

In 1896 in a field of beets covering an area of about one acre and containing approximately 130,000 beets, eleven badly diseased and several slightly affected ones were found. This was a smaller number than had been found on the same ground in previous years, and can perhaps be accounted for by the climatic conditions being so favorable to plant growth the preceding summer, there being an abundance of rain. The number of diseased beets increased, however, toward harvest time.

Frost seems to be much more injurious to the diseased than to the healthy beets. The heart leaves of the diseased beets were more easily injured by the frost. It is characteristic of the disease that the leaves of badly diseased roots die away until no green leaves remain, leaving an apparently dead root in the soil, though its tissues will be found to be firm and not in the least broken down. The early frosts hasten the destruction of the leaves. Both diseased and healthy roots show an acid reaction, the diseased seeming slightly more acid than the healthy.

ETIOLOGICAL HISTOLOGY.

Comparative study was made of sections taken from both diseased beet roots and healthy beet roots, also sections from leaf and leaf-stalk of both diseased and healthy beets. In all these sections small round bodies were seen in the cell substance. These bodies were found to be protein by their reaction to iodine. They measured from 2–4μ in diameter and turned yellow when treated with iodine. In the tissues of diseased beets other bodies were found which were smaller and of a different refractive power and arrangement. These bodies stained in gentian violet like bacteria, and looked almost like micrococci when imbedded in the cell substance, but when free in the water were easily distinguished as small motile bacilli.

SEPARATION OF THE SPECIFIC GERM.

The first steps in the separation of the germ were as follows. A diseased beet was selected, a thin knife sterilized in the flame and used to remove all parts of the beet exposed to the air. A small piece of beet was then removed from the heart of the root and transferred by means of a sterilized platinum wire to tubes of melted gelatin or agar. The first series of cultures was made by inoculating gelatin tubes with pieces of diseased tissue, at the same time inoculating a number of tubes in the same manner with pieces of healthy beet as a control. The following tables give the results of a series of such inoculations. The first shows the results of cultures made from diseased tissue; the second the results of cultures made from healthy tissue.

TABLE I.

CULTURES ON ARTIFICIAL MEDIA FROM DISEASED AND HEALTHY BEETS.

Dates and media	Number of cultures	Results	
		FROM DISEASED BEETS.	
Sept. 20 Gelatin - -	10	Oct. 5 Characteristic growth	Oct. 10 Contaminated Dec. 10 Still pure
Oct. 2 Gelatin - -	3	Oct. 4 Characteristic growth	
Oct. 24 Glycerin gelatin	4	Oct. 28 Characteristic growth	
Oct. 24 Glucose gelatin	4	Oct. 28 Characteristic growth	
		FROM HEALTHY BEETS.	
Sept. 20 Gelatin - -	4	Oct. 5 No growth	Oct. 10 No growth Dec. 10 No growth
Oct. 2 Gelatin - -	3	Oct. 4[5] No growth	
Oct. 24 Glycerin gelatin	4	Oct. 28 No growth	
Oct. 24 Glucose gelatin	0		

All the above cultures were made in standard gelatin not [illegible]ted or in standard agar to which had been added 5 per cent glycerin or 5 per cent. glucose. The growth in successful inoculations was the same in all cases. Some creamy-white globules grew out on either side of the diseased tissue, and in the course of a few days were surrounded by a lens-shaped capsule or break in the gelatin caused by the gas that was given off in the growth. When the bubbles reached the surface the growth was distributed in white rings around the tube (*plate XX, B″*).

October 15 several cultures were made in standard gelatin. In some of these a growth resulted but no successful transfers were made. One of these cultures was used to inoculate a healthy beet in the field.

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[5] Photograph of tubes at this stage shown in *plate XX*.

November 3 stab cultures were made from a tube of glycerin agar inoculated with diseased tissue. These were all contaminated with the exception of one which formed a perfectly colorless layer, gelatinous in consistence, on agar and sterilized beet. This form will be spoken of later.

No growth resulted from a series of cultures made in 10 per cent. cane sugar gelatin.

In December a diseased beet, which was frozen the previous night, was brought in from the field. Pieces of this tissue were transferred with the usual precautions to tubes of melted gelatin to which had been added 5 per cent. of cane sugar. The growth in these cultures was rapid and gas was given off in large quantities. Stab cultures were made from these and appeared exactly uniform, and just the same in appearance as stab agar cultures made directly from unfrozen diseased beet, with one exception in each case. From one of these exceptional tubes the perfectly colorless gelatinous form spoken of above was found. This form was also obtained in one of the stabs taken directly from the diseased beet.

In the above inoculations the growth was much more rapid than in previous cultures, probably because the tissues were broken down by freezing so that the germ could escape more easily into the surrounding medium.

Another series of cultures was made at the same time from a frozen beet in which the disease was produced by inoculation. The growth was similar to that of the preceding series. Transfers taken from these were uniform and similar to those described above.

In these inoculations a plug was removed from the healthy beet root with a sterilized knife, the inoculating material inserted, the plug replaced and covered with cotton. The table on the next page shows the results of inoculations of three beets in the field. The beet inoculated with diseased tissue was slower in showing the disease than the one inoculated with the germ growing on gelatin, probably because of the time required for the germ to make its way through the cell walls of the tissue.

TABLE II.

RESULTS OF INOCULATING BEETS IN THE FIELD.

Beet	Date of inoculation	Source of germ	October 22	November 19	November 28
1	Oct. 17	Isolated germ	Yellowish color of leaves noticed	Disease quite evident	Removed from the field
2	Oct. 17	Diseased tissue	Slight indication of the disease	Disease quite evident	Removed from the field
3	Oct. 17	Healthy tissue	No change	No change	No change

The heart leaves of the inoculated beets showed the effect of the disease in the slight blister-like areas on their surface. The beet inoculated with the gelatin containing the germ, and the one inoculated with healthy tissue, were brought into the laboratory November 28, as the progress of the disease could not be followed out of doors because of injury by frost. The beets were placed in culture jars of water in the greenhouse where the healthy beet after some time decayed, and the diseased beet developed new leaves which were more or less crinkled and faded, but gradually assumed a smoother and darker green appearance, but the plant was still stunted in growth.

INOCULATIONS IN THE GREENHOUSE.

The method of inoculation of beets in the greenhouse was similar to that of inoculation of beets in the field. As a result of these trials several beets seemed to show the effects of the disease to a slight degree.

DESCRIPTION OF THE GERM.

The germ as isolated from the diseased tissue is a small bacillus measuring from 0.9–1.0–1.3 μ in length, and 0.5–0.8 μ broad. When taken from the culture media the germs are arranged singly or in pairs, and possess individual motion. The germ seems to revolve more or less irregularly on its axis. The germ stained well with all the common bacterial stains. No process of staining showed the presence of spores or flagella (*plate XX, B*).

EFFECT OF LIGHT ON GROWTH.

The germ grew better in the dark than in the light. Germs taken from old, dried out cultures were smaller than when grown on a moist substratum. Desiccation also injures the capability of the germ for motion. Germs taken from an old culture and examined in a drop of water were less motile than those taken from a fresh bouillon culture.

The germ grows better at a temperature of 12°–14° than at 21°. Stab cultures in agar grew slowly at body temperature. The germ grown in bouillon and exposed to a temperature of 100° for five minutes was killed.

GROWTH OF THE GERM ON DIFFERENT CULTURE MEDIA.

Stab cultures of gelatin showed a thin grayish-white layer on the surface, and extending down the line of inoculation. As the cultures grew older the color darkened to a deep cream. The gelatin was liquefied in the course of several weeks. When melted gelatin was inoculated and then allowed to solidify there was a growth throughout the gelatin in streaks and films. Colonies on gelatin plates were not distinctly outlined and were sometimes accompanied by a disagreeable odor. The germ seemed to grow better on agar than gelatin. Agar to which had been added 3 per cent. of cane sugar or glucose seemed to specially favor its growth. The growth on slant agar was drab-white in color, smooth margins, and a slow and not luxuriant growth. The growth was not viscous.

In agar plates the colonies have their origin in the deeper layers of the agar where they are generally elliptical. When they reach the surface they spread out in their round grayish-white colonies with compact creamy-white centers. In bouillon growth is observed after two or three days. No turbidity of the fluid was observed, but a sediment was deposited in the bottom of the tube. Masses of zooglœa were sometimes found in old bouillon cultures. The germ grew well on sterilized sugar beet, and deposited a sediment in sterilized beet juice. The germ grew on sterilized apple, potato, and turnip. A raw potato was broken open and inoculated with the germ. There was a slight growth developed. A raw sugar beet was inoculated. The germ grew, causing a black coloration of the fibrovascular bundles, and in a microscopical examination was seen to have entered the tissue.

NITRATE SOLUTION.

This solution was prepared using 1000^{cc} distilled water, 1 gram peptone, and 1 gram potassium nitrate. Tubes of this solution in which the germ had grown for three days when tested showed that the nitrate had been fairly well reduced.

ACID AND ALKALINE MEDIA.

It has been stated that the beet root is acid to the extent of little over 1 per cent. Because of this fact experiments were made with acid and alkaline media in order to determine which of the two would be more favorable to the growth of the germ. In bouillon, to which had been added 1 per cent. malic acid, the solution was made neutral; 5 per cent. acid solutions were not rendered neutral. The solution was not made turbid by the growth of the germ. In 1 per cent. alkaline solution of bouillon the germ was more motile than in 1 per cent. acid solution. In 3 per cent. alkaline solutions the germ was more motile and larger than when grown in acid media, measuring from 1.1–1.9 μ long, and 0.9–1 μ broad. In 5 per cent. alkaline media the sediment deposited was quite viscous. Zooglœa masses were found more or less abundantly in all the alkaline cultures. These solutions were not made acid in reaction by the growth of the germ.

STARCH SOLUTIONS.

In a solution composed of one part each of starch filtrate and bouillon, the germ grew but the starch was not reduced. These cultures were tested for starch and gave a decided reaction; they were also tested with Fehling's solution for glucose, but gave no reaction. The germ grew well in wort gelatin.[6] When this gelatin was melted, and after being inoculated was allowed to solidify, the germ grew under the surface, producing bubbles of gas all through the gelatin.

Tests made of bouillon containing cane sugar in which the germ had grown gave a reaction for glucose. In order to determine if the enzyme existed outside the cell, a solution in which the germ had grown was filtered through a porous cup. This filtrate added to a 5 per cent. cane sugar solution, and tested with Fehling's solution for glucose gave no reaction. Further experiments are necessary before deciding definitely in regard to the enzyme properties of the germ.

CELLULOSE SOLUTIONS.

As the germ penetrates the cell wall of the plant in some way, experiments were made to determine its effect on cellulose. For this a special nutrient solution was prepared as follows:[7]

Distilled water,	250	cc.
Pepsin,	2.5	gram.
Magnesium sulfate,	.45	"
Calcium phosphate,	.45	"
Ammonium sulfate,	2.05	"
Sodium chlorid,	1.25	"
Beef extract,	1.25	"
Swedish filter.		

In this solution the growth was very slow, and a very small amount of gas was produced, and the cellulose slightly broken down.

FERMENTATION.[8]

Because of the abundance of gas produced by the germ in its growth, special fermentation solutions were prepared. The gas produced by the growth of the germ in these solutions was analyzed by Mr. H. S. Bryan, of Purdue University, the results of which are given in Table III. The tests were made for CO_2, O, N, NH_4, and CO, the difference being considered as hydrogen. In the first analysis a much larger amount of nitrogen was obtained than in any of the other cultures. The cultures for this analysis were several weeks old, and were perhaps not trustworthy.

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[6] Wort gelatin was made by adding 10 per cent. of gelatin to wort.

[7] Sur la fermentation de la cellulose. Centralblatt für Parasitenkunde **2**:358. 1896.

[8] Pammel, L. H. and Emma: A report concerning gases produced by bacteria fermentation. Centralb. f. Parasitenkunde **2**:707. Dec. 1896.

There were some irregularities in the amount of gas produced, which cannot be accounted for. At one time 2 per cent. cane sugar bouillon containing no pepton when inoculated gave a large amount of gas, 20^{cc} being collected in each fermentation tube; 2 per cent. cane sugar bouillon containing no pepton, inoculated at another time under exactly similar conditions, gave only 2^{cc} of gas in each fermentation tube.

The germ grown in bouillon to which had been added 2 per cent. glucose at one time gave a very large amount of gas; at another time there was not enough gas produced to be analyzed. The gas produced by the germ as determined by analyses is composed of a very small amount of oxygen, less than 4 per cent., carbon dioxide approximately 44 per cent., nitrogen 17 per cent., and hydrogen approximately 30 per cent. Fermentation was produced in sterilized beet juice, Pasteur's solution, and maple sap. No fermentation was produced by the growth of the germ in bouillon to which no sugar had been added.

SUMMARY.

It has been determined that a microscopical examination of the tissues of diseased beets reveals the presence of bacteria in the cells of the plant. The tissues of the plant are not broken down, and the bacteria in all parts of the plant appear to be the same. Transfers of diseased tissue to the healthy beet root resulted in changed appearances of the plant which indicated almost certainly that the disease was transmitted.

The manner in which the germ finds entrance to the plant has not been determined. The conditions most favorable to the

TABLE III.

ANALYSIS OF THE GAS PRODUCED BY THE SUGAR BEET GERM.

Date of Inoculation	No. of tubes	Solution	Amount of gas	Results of analysis				
				CO_2	O	H	N	NH_4 and CO
Dec. 18	3	5 % cane bouillon	Dec. 21. 4^{cc}	25%	a trace	a trace	50–60%	none
Jan. 18	several	2 % cane bouillon	Jan. 22. 20	67	.5%	20.6%	11.9	"
Jan. 25	3	2 % cane bouillon	Jan. 29. 15	61.8	.69	27.5	10.1	"
Jan. 25	3	2 % glucose bouillon	Jan. 29. 20	38.7	3.6	36.5	21.2	"
Jan. 25	2	2 % lactose bouillon	Not enough gas to	be analyzed				
Jan. 25	3	2 % cane bouillon, no pepton	Feb. 2. 20	37.63	7.22	21.64		"
Feb. 3	7	2 % glucose bouillon	Feb. 11. 2	18	1.7	explosion probably H	33.51	"
Feb. 3	3	2 % cane bouillon, no pepton	Feb. 11. 2	No analysis	made			
March 29	several	5 % cane bouillon, with 3 % calcium chlorid	April 26 10	44.23	1.92	33.65	14.02	"
March 29		7 % acid wort solution	April 26 15	53.27	.9	31.77	14.09	"
April 1	several	Cellulose solution	. Small	No analysis	made			

attack are those resulting from drought with succeeding low temperature.

The fact that the germ breaks down cellulose slowly explains the manner of its progress from one cell to another.

Experiments have shown that the germ in a medium of low per cent. acid grows nearly or quite as well as in one of alkaline nature, so that the acid element of the beet root does not offer material resistance to the germ.

The germ converts cane sugar to glucose in the process of producing gas. The amount of gas produced is not constant, but the reasons for this irregularity have not been determined.

The germ grows well with any form of sugar and especially well in media containing cane sugar. This fact makes it seem probable that the germ is especially at home on those media which contain sugar in some form, although it will keep alive on media without sugar, and after cultivation for a time on such media will adapt itself to the conditions presented.

ANOTHER ORGANISM SEPARATED FROM THE SUGAR BEET.

The colorless gelatinous form separated from the beet root in connection with the disease germ was at first thought to be an undescribed germ or rather the product of a germ, for only a few bacterial bodies could be detected under the microscope even when comparatively large masses of the substance were placed in the field. The organism appeared as small bacilli or micrococci.

The mass resembles the form of Leuconostoc so common in the vicinity of sugar refineries. Under the microscope, however, no streptococci were found, which characterizes Leuconostoc under the microscope. The gelatinous substance is soluble in water and alcohol; in the latter it turns to a milk-white substance before it dissolves. The substance increased rapidly in bulk when grown on sterilized beet. The mass did not dry out for months after the substratum had become dry and hard.

The substance grew well on 10 per cent. cane sugar agar. The growth was slow at first, but after a week or two masses measuring a quarter of an inch in thickness and three fourths of an inch in circumference, collected on the surface of the medium in stab and slant cultures. In case of stab cultures the agar was broken vertically along the line of inoculation. The colorless growth followed this break in the agar, and as the substratum became hard the mass collected as a colorless semi-fluid in the bottom of the test tube.

On slant agar there was a thin colorless layer, imparting a fluorescent hue to the medium. In agar plate cultures the organism formed small round colonies about the size of a pin head, resembling a small drop of water. These colonies were sometimes found with the disease germ, in plate cultures taken directly from the beet. It also grew on sterilized potato, and to some extent on gelatin. Immediately after separation from the beet root the organism produced fermentation, but the power was lost after a time. Staining revealed only a structureless mass containing a few bacteria-like bodies.

Desiccation has little effect on the substance. Sections of beet on which the organism was growing have been kept in the laboratory until they are quite dried out, and the gelatinous mass is still apparent.

If this is indeed a form of Leuconostoc, it is interesting to find it in diseased beet roots.

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EXPLANATION OF PLATES XVI–XX.

PLATE XVI.

Leaves of healthy and diseased beets, as they appeared when brought in from the field. The three diseased leaves can readily be distinguished from the two healthy ones because of their blistered and crinkled surface.

PLATE XVII.

A diseased beet brought in from the field. The root is quite firm, none of the tissue being broken down. The leaves hanging down are quite dead and dry. The erect heart leaves are alive and show the characteristic crinkled surfaces.

PLATE XVIII.

A, a diseased beet with the dead leaves removed. *B*, a healthy beet of same size and stage of growth, also with the dead leaves removed.

PLATE XIX.

A, cross sections through the crown of healthy and diseased beet roots. *a*, healthy root. *b*, diseased root, characterized by the black rings of vascular tissue.

B, cross sections through the central portion of the same diseased and healthy beet roots. *a*, healthy root. *b*, diseased root.

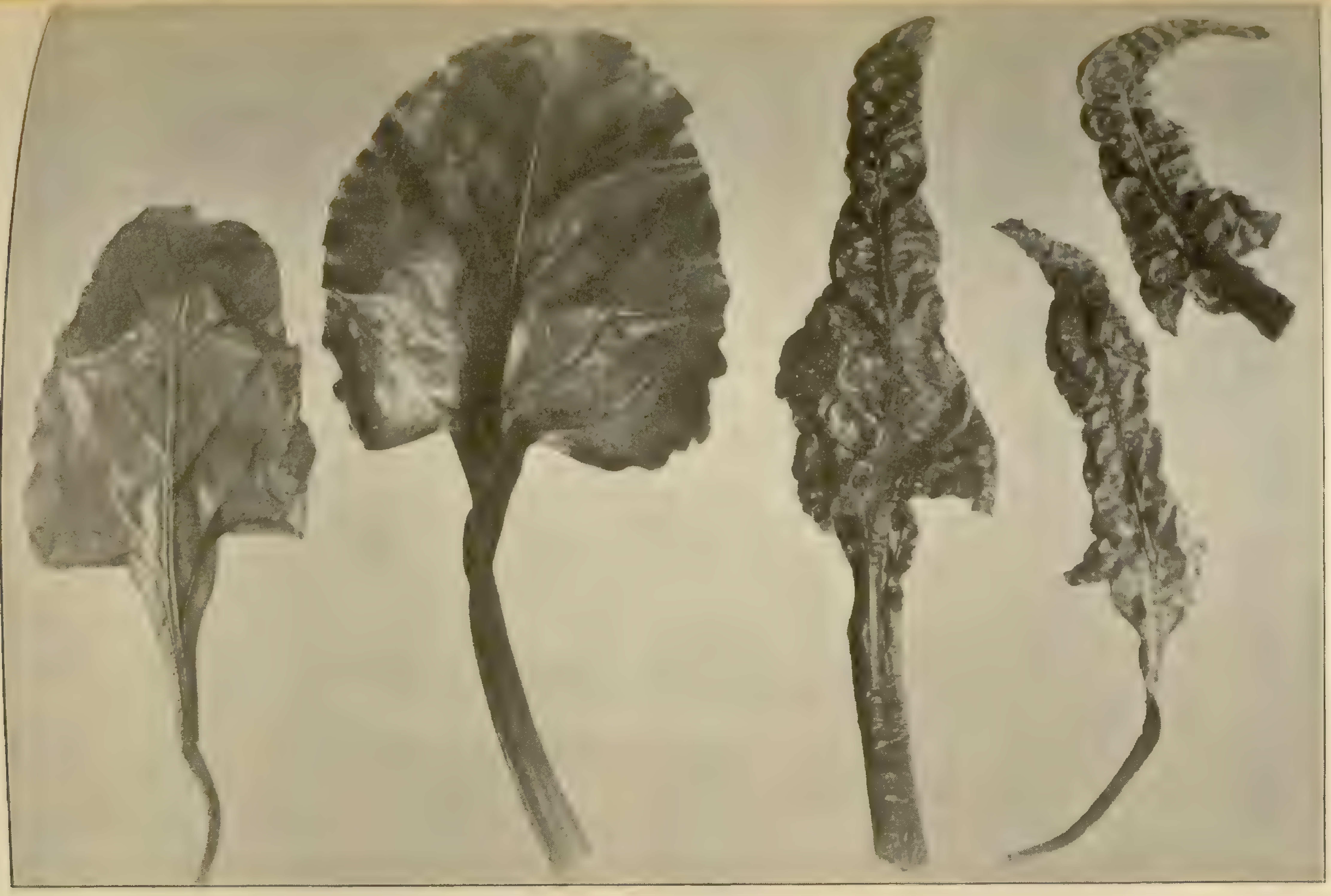
C, cross sections near the tip of the same roots. *a*, healthy root. *b*, diseased root.

PLATE XX.

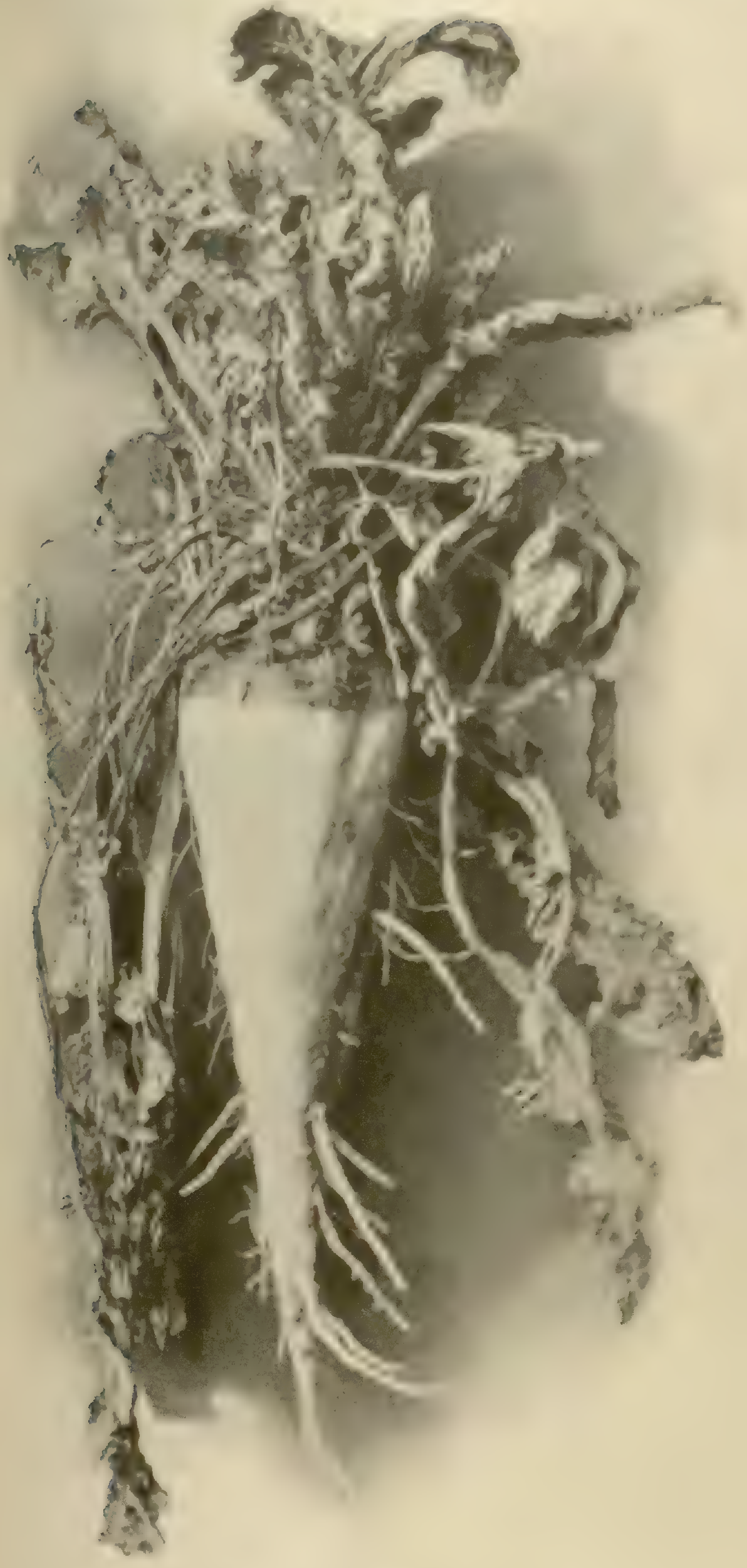
A, longitudinal sections of the same healthy and diseased beet roots figured in the preceding plate. *a*, healthy beet. *b*, diseased root.

A″, tube of gelatin inoculated with a piece of healthy beet. *B″*, similar tube inoculated with a piece of diseased tissue. The photographs were taken after being inoculated two weeks. In *A* no growth appeared. In *B* the small globules of growth can be seen, breaking the agar and pushing it upward by the production of gas.

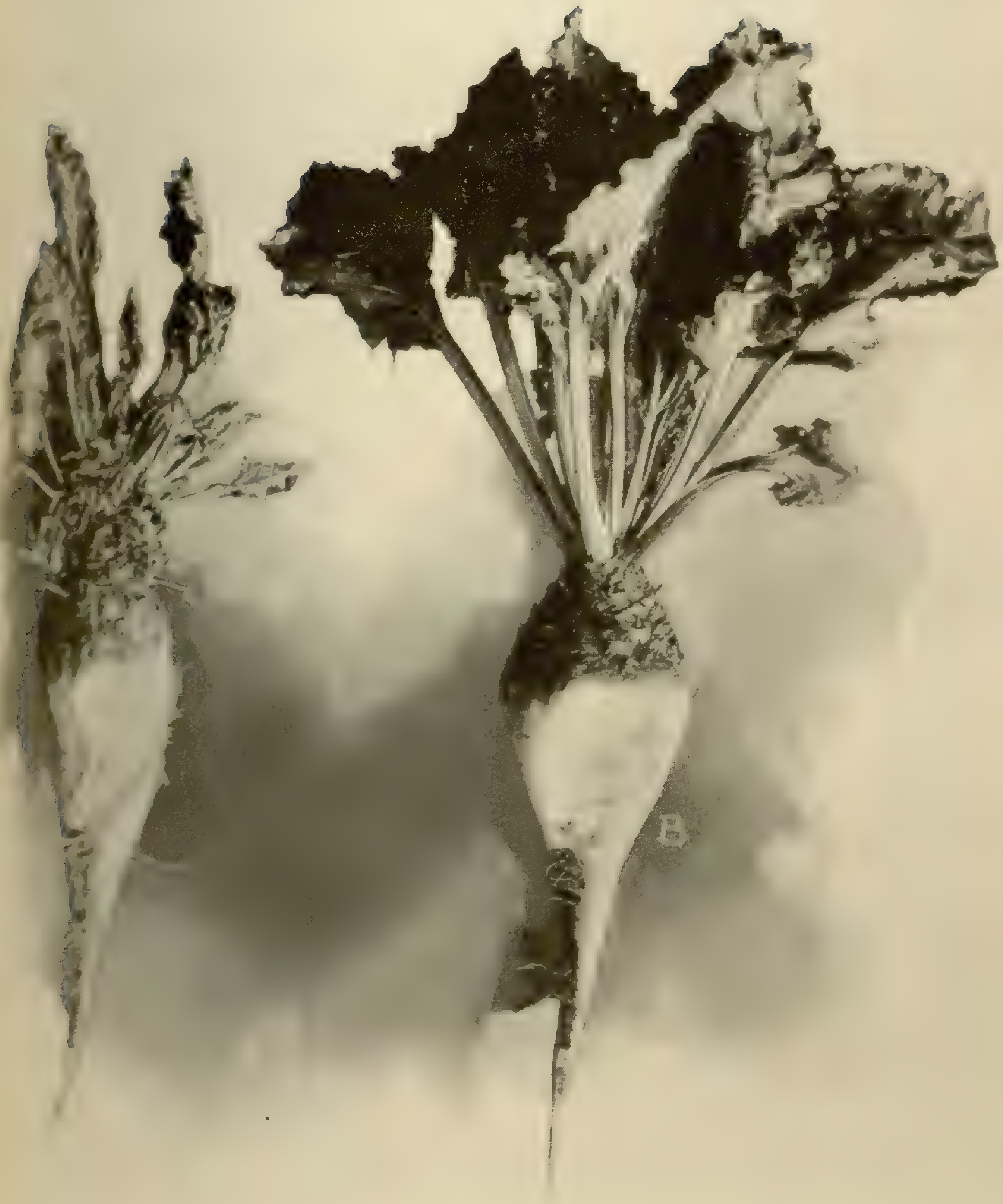
B, the disease germ stained with carbol fuchsin; imperfectly photographed.



CUNNINGHAM on SUGAR BEET



CUNNINGHAM on SUGAR BEET



CUNNINGHAM on SUGAR BEET

CUNNINGHAM on SUGAR BEET

CUNNINGHAM on SUGAR BEET

REVISION OF THE NORTH AMERICAN SPECIES OF TEPHROSIA.

B. L. Robinson.

While the greater part of the difficulties of the genus Tephrosia lie, happily, beyond the geographical limits of the North American flora, yet the dozen species which inhabit sandy regions in our southern states are sufficiently variable in their foliage and similar in their floral and carpological structure to lead to diverse views on their specific limits and proper arrangement. Miss Vail's recent monograph[1] of these species, although abounding in long and detailed descriptions, fails to bring out clearly the primary divisions of the genus. The introductory synopsis—which, although arranged in perfect accord with the natural sequence of the species in the paper, is styled an "artificial key"—is based chiefly upon the length and density of the inflorescence, features which are too variable and confluent to furnish diagnostic characters of the first rank. It seems to the writer that the sympodial and monopodial structure of the stem offers a difference much more evident and, indeed, sufficiently fundamental to divide our species at once into two well-marked sections. After this first division the size of the flowers, density of the inflorescence, and nature of the foliage may be employed with greater diagnostic effect.

In the limitation of species it is believed that Miss Vail's *Cracca floridana* is only separated artificially from her *C. Smallii*. The distinctions adduced are chiefly the "truly prostrate" habit of the former species, and its narrower, more numerous leaflets. As to the breadth of the leaflets, this is (according to Miss Vail) 6 to 14^{mm} in *C. Smallii*, and 5 to 12^{mm} in *C. floridana*, surely no very striking difference. In number the leaflets are said to vary in *C. Smallii* from 3 to 11, while in Mr. Nash's authenticated specimens of *C. floridana* they vary from 7 to 13, and had these specimens been younger, it is safe to surmise, leaves with fewer leaflets would have been found, an inference amply supported by the development in related species. Regarding the supposed assurgent habit of *C. Smallii* (a species not only distinguished in the field, but called to scientific notice by Mr. A. H. Curtiss), it may be said that the main stems bear evidence of being prostrate except near the tip. This is shown not only by the curvature and position of the leaves and peduncles, but by the almost constant presence of clinging particles of sand which adhere, even in the type specimens, to the lower surface of the stem, and are lacking upon the upper surface. This position of the stem is fully confirmed by field notes kindly furnished by Mr. Curtiss. The assurgent character is, therefore, confined to the leaves (which are said to be somewhat ascending but less erect than in *T. ambigua*), the peduncles, and the growing tips of the stems. On the other hand, specimens of *C. floridana*, although said to have prostrate stems and leaves, give by no means the impression of a closely prostrate plant, an idea which is at once conveyed by specimens of *T. chrysophylla*. In the absence of more telling morphological features the two species *C. floridana* and *C. Smallii* are here united.

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[1] A revision of the North American species of the genus Cracca. Bull. Torr. Bot. Club 22: 25–36.

Miss Vail is certainly in error in interpreting Shuttleworth's *T. angustissima*, her characterization evidently being drawn from Mr. Curtiss' plants so named. Shuttleworth's type, collected on the Miami river by Rugel, is in the Gray herbarium, and is clearly a linear-leaved form of *T. purpurea* Pers., with which it shares the characteristic small flowers, of which one or two are axillary, and the rest borne in a very slender raceme. On the other hand Curtiss' nos. 584 and 5708, just mentioned, appear to be nothing but smoothish narrow-leaved *T. ambigua*, not differing by a single morphological character of specific value. In her key (p. 26), Miss Vail distinguishes this plant (her *Cracca angustissima*) from *T. ambigua* on the ground that it is *erect*, and has linear leaflets, but in her description (p. 32) it is characterized as *prostrate*.

T. Rugelii Shuttleworth is an interesting and hitherto undescribed species possessing the stem-structure of § BRISSONIA but the habit and foliage of *T. chrysophylla*, which is of § REINERIA.

T. tenella Gray seems to have been founded upon a juvenile state of *T. purpurea*, to which (under the name of *T. leptostachya* DC.) it was reduced by Bentham in Mart. Fl. Bras. 15^1: 48. *T. purpurea*, however, is highly variable, at least in foliage, and certainly merits further study with more copious material than is now available.

The writer is grateful to Drs. Britton and Small for the loan of the larger part of the North American Tephrosias from the herbarium of the New York Botanical Garden. These have been of material assistance in the present revision.

As the genus is here interpreted in its generally accepted significance, it is useless to take space for a generic description. Besides the species here described, the tropical *T. cinerea* Pers. has been found in the southern states, but only on ballast ground (Alabama, *Dr. C. Mohr*). It is of the § REINERIA, and has rather numerous narrowly oblanceolate-linear leaflets, which are hoary-pubescent upon both surfaces.

§ 1. BRISSONIA DC. Stem monopodial : racemes terminal or axillary, never opposite the leaves : flowers (in our species) large, with petals 1 to 1.7^{cm} long.—Prodr. **2** : 249, in part ; Taubert in Engl. & Prantl, Pflanzenf. **3** : Ab. 3. 269. *Brissonia* Necker, Elem. **3** : 36.

* Pods glabrous at maturity : racemes loose : southwestern.

T. LEIOCARPA Gray. Undershrub, cinereous with fine appressed pubescence : stems several, 4 to 6^{dm} high, suberect : leaves petiolate, 15–19-foliolate ; leaflets narrowly oblong, 1.6 to 3.2^{cm} in length : racemes both terminal and axillary : pods 5 to 5.7^{cm} long, about 10-seeded.—Pl. Wright. **2** : 36 ; Walp. Ann. **4** : 489. *Cracca leiocarpa* Kuntze, Rev. Gen. **1** : 175 ; Vail, Bull. Torr. Club 22: 28 (where type locality is erroneously stated to be New Mexico).—Sonoita valley, close to the southern boundary of

Arizona, *Wright*, no. 965, *Rothrock*, no. 685. (Northern Mexico.)

* * Pods permanently pubescent or puberulent: inflorescence short and dense.

+ Calyx-lobes ovate-lanceolate to lanceolate: pubescence gray: petioles rarely 6^{mm} in length.

T. VIRGINIANA Pers. (GOAT'S RUE.) Stems several, 3 to 4.5^{dm} high, erect from a stout knotted lignescent root: pubescence fine, soft, somewhat variable in quantity, often copious toward the summit: leaflets 11 to 23, oblong to elliptical, green and scarcely pubescent above, somewhat paler and soft villous beneath, 2.5 to 3.2^{cm} long; petioles very short; stipules caducous: flowers borne partly in pairs or singly in the upper axils but chiefly in a short dense raceme little raised above the surrounding leaves: calyx hairy, the teeth caudate-acuminate: petals white or pale yellow with purplish tinge: hirsute pods soon spreading or divaricate.—Syn. **2**: 329; Pursh, Fl. **2**: 489; Ell. Sk. **2**: 245; Torr. & Gray, Fl. **1**: 295; Wats. & Coulter in Gray. Man. ed. 6, 133; Meehan, Nat. Fl. **1**: 81. *pl. 21.* *T. virginica* Bigel. Fl. Bost. ed. 3, 296. *Galega virginica* L. Spec. ed. 2, **2**: 1002; Hill. Veg. Syst. **21**. *pl. 55, f. 1.* *Cracca virginiana* L. Spec. **2**: 752; Vail, l. c. 27.—Dry open woods especially in sandy soil, common; New England to the north shore of Lake Erie, thence to Texas and Florida; fl. May, June; fr. July to September.

Var. HOLOSERICEA Torr. & Gray. Leaflets inclining to be narrow and acute; pubescence more copious, long and silky or even woolly on the pods.—Fl. **1**: 296. *T. holosericea* Nutt. Jour. Acad. Philad. **7**: 105. *Cracca virginiana* var. *holosericea* Vail. l. c.—Arkansas, *Nuttall*, *Marcy Exp.*, and Texas, *Hall*. Similar but less marked forms in Wisconsin and W. New York.

+ + Calyx-lobes very narrow, almost filiform: pubescence tawny.

++ Petioles 1.7 to 4.2^{cm} long: leaflets oblong: southwestern.

T. LEUCANTHA HBK. Erect, branched from a lignescent base: leaflets 15 to 20, oblong, appressed-villous on both surfaces, nearly concolorous, 1.9 to 3.2^{cm} long: racemes capitate, pedunculate, chiefly terminal; linear filiform bracts considerably exceeding the buds: petals white with or without a purple tinge: pods narrow, 5 to 6.2^{cm} long, soon divaricate.—Nov. Gen. & Spec. [illegible] *pl. 577;* Gray, Pl. Wright. **2**: 36; Torr. Bot. Mex. Bound. [illegible] *Cracca leucantha* Kuntze, l. c.; Vail, l. c.—Mountainous [illegible] in S. Arizona, *Rothrock*, *Lemmon*, *Pringle*. (Mex. where first collected by *Humboldt & Bonpland.*)

++ ++ Petioles 4 to 17^{mm} long: leaflets obovate: Florida.

T. Rugelii Shuttleworth in herb. Stems several, decumbent or suberect from a lignescent stock, finely appressed-pubescent with bronze-colored hairs: leaves 3–11-foliolate; stipules persistent, 4 to 6^{mm} long; leaflets obovate, retuse, mucronulate, finely appressed-pubescent and yellowish-green above, decidedly paler, cinereous and very veiny beneath, 8 to 17^{mm} long, half as broad: flowers borne chiefly in pairs in the upper axils or forming a subcapitate raceme at the summit of the stem: calyx tawny-villous, 5^{mm} long, its narrow teeth subequal: petals probably purple: pods somewhat falcate, 3.8^{cm} long, 5^{mm} broad, tomentulose.—In pine woods on the Manatee river, S.W. Florida, *Rugel*, no. 156, June 1845. Type in herb. Gray. A characteristic and, according to our present knowledge, thoroughly distinct species with the habit of *T. chrysophylla*, from which it differs in its monopodial stem and axillary flowers, as well as in the presence of pubescence upon the upper surface of the leaves.

§2. Reineria DC. l. c. 251, in part. Stem sympodial, the main axis at one or more nodes terminating in a raceme which by the strong development of an axillary bud at its base becomes apparently lateral: some of the racemes thus appear to arise opposite the leaves. (The sympodial structure is tardily developed in *T. purpurea*, which during its first season sometimes produces only a terminal raceme. This species, however, may be readily distinguished from those of the preceding section by its smaller flowers, which are only 6 to 8.5^{mm} long.)—*Reineria* Moench, Meth. Suppl. 44.

* Flowers large: petals 1 to 1.7^{cm} long.

+ Leaflets (with rare exceptions) exceeding the short petioles.

+ + Flowers not numerous, borne singly or in pairs at the nodes of the racemes.

= Stem covered at least below with a short dense bronze-colored tomentum: leaflets thickish, of firm or subcoriaceous texture, glabrous and finely reticulated above.

a. Leaves prostrate, essentially sessile; leaflets seldom more than 7.

T. CHRYSOPHYLLA Pursh. Perennial herb with spreading prostrate freely branched somewhat flexuous or geniculate stems, subsessile, 2–7-foliate leaves, and obovate leaflets (1.3 to 3^{cm} long): few-flowered racemes opposite the leaves; peduncles scarcely ancipital, 5 to 8.8^{cm} long: petals white, changing to red: pubescence on the lower surface of the leaflets dense, sericeous, somewhat canescent but with a slight golden sheen: pods 3.4 to 4.2^{cm} long, 8–10-seeded.—Fl. **2**: 489; Ell. Sk. **2**: 246; Torr. & Gray, Fl. **1**: 297; Chapm. Fl. 95. *T. prostrata* Nutt. Gen. **2**: 120. *Cracca chrysophylla* Kuntze, l. c. 174; Vail, l. c. 34.—Dry pine woods, Georgia, *Boykin*, *Forbes*, to Florida, where apparently common, "and westward" acc. to Chapman, but probably in reference to *T. Smallii.*

Var. **Chapmanni.** Plant smaller, leaflets 6 to 13^{mm} long, half as broad: pods only 1.9^{cm} long, 5–8-seeded.—*Cracca chrysophylla* var. *Chapmanni* Vail, l. c.—St. Josephs, Florida, *Dr. Chapman.*

b. Leaves, at least in some cases, ascending, petiolate; leaflets mostly 7 to 11.

T. **Smallii,** n. comb.—Similar to the preceding species in many ways, but stouter, with more numerous and longer (oblong or elliptic rather than obovate) leaflets: peduncles decidedly ancipital above, becoming 5 to 20^{cm} in length.—*Cracca intermedia* Small, Bull. Torr. Club. **21**: 303. *C. Smallii* and *C. floridana* Vail, l. c. 33, 35.—Pine barrens in sand, Georgia, *Boykin*, to Florida, *Curtiss*, *Nash*, and Louisiana, *Dr. Ingalls*. The previous use of *intermedia* in Tephrosia necessitates the adoption of the second specific name.

= = Stems very slender, sparingly pubescent: leaflets rather small, elliptical, thin.

T. HISPIDULA Pers. l. c. Stems several, branched, spreading and ascending from a thickish somewhat fusiform root, finely pubescent or glabrate; hairs sometimes spreading but usually appressed: leaflets 11 to 17, elliptic-oblong to linear-lanceolate, thinnish, usually deflexed, appressed-pubescent or glabrous above, slightly paler appressed-villous beneath, usually rounded and mucronulate at the apex: petals at maturity purple, sometimes 1.6^{cm} in length: pods 8–10 seeded, covered with short appressed or more often spreading hairs.—Pursh, Fl. **2**: 489; Ell. Sk. 2: 245; Torr. & Gray, Fl. **1**: 296, excl. vars.; Chapm. Fl. 95. *T. gracilis* Nutt. Gen. **2**: 119. 1818. *T. elegans* Nutt. Journ. Acad. Philad. **7**: 105. *Galega hispidula* Michx. Fl. **2**: 68. *Cracca hispidula* Kuntze, l. c. 175; Vail, l. c. 33.—Sandy barrens, Virginia and N. Carolina, *Curtis*, to Florida and Louisiana, *Hale*.

Stems rather stout, covered with a copious coarse tawny spreading pubescence; leaflets sparingly villous along the midnerve above or appressed-pubescent over the entire upper surface.

T. VILLOSA Pers. l. c.[1] Stem 3 to 9^{dm} long, sprawling, tawny-hirsute: leaflets 3 to 17, elliptic or obovate, rounded and apiculate at the end, villous beneath, more or less appressed-pubescent also on the upper surface, about 2.5^{cm} long, a third to half as broad; rhachis tawny-pubescent: peduncles long, somewhat sincipital; interrupted few-flowered raceme surpassing the leaves: calyx lobes with long filiform tips: petals pale or more often (at least in age) purple red.—*T. spicata* Torr. & Gray, Fl. **1**: 296; Chapm. Fl. 95; Wats. & Coulter in Gray, Man. ed. 6, 133. *T.*

[1] This species was originally described by Michaux (1803) as *Galega villosa*, with a range "a Carolina ad Floridam." There is absolutely no evidence that Michaux regarded his plant as the equivalent of the Asiatic *G. villosa* L. The name *Tephrosia villosa* is first employed by Persoon in his Synopsis (1807) and is there used exclusively for the American plant. This is shown not only by the identical range but by the quoted character, which is taken bodily from Michaux's work. The usage of De Candolle and many more recent European writers, by which the name *T. villosa* Pers. is applied to an East Indian and African species, to which Persoon's description had no reference whatever, is clearly an unwarrantable transposition. It is true that there was an earlier *Galega villosa* than that of Michaux, but this should not invalidate *Tephrosia villosa* Pers., which is clearly applied to the American plant and invalidated by no homonym. The plant of the Old World, although possessing an earlier specific name, was not brought under Tephrosia until later and, it is believed, should in that genus receive another specific designation.

paucifolia Nutt. Gen. **2**: 119. ? *T. hispida* DC. Prodr. **2**: 25 *T. mollissima* Bertol. Bot. Miscel. 9: 10. *pl. 3. f. 2*, and Bot. Zeit. **9**: 902, acc. to Gray. *Galega spicata* Walt. Car. 188. *G. villosa* Michx. Fl. **2**: 67. *G. paucifolia* M. A. Curtis, Jour. Bost. Nat. Hist. Soc. **1**: 122. ? *Crafordia bractiata* Raf. Specch. **1**: 15 *Cracca spicata* Kuntze, l. c.; Vail, l. c. 30.— Dry sandy ground, common, Delaware to Florida, west to Arkansas (acc. to Lesquereux) and Louisiana, *Hale;* fl. May to July.

— Var. **flexuosa**. Leaflets linear to lance-linear, acute, the terminal one much elongated.— *T. flexuosa* Chapm. acc. to Torr. & Gray, l. c. 297, in synon. *T. hispidula* var. γ Torr. & Gray, l. c. *Cracca spicata* var. *flexuosa* A. M. Vail, l. c.— Florida, *Chapman*; poorly known and perhaps distinct. A similar but nearly glabrous form has been found in Alabama by *Gates*.

++ ++ Flowers numerous, the middle ones borne in threes and fours: leaflets also numerous, 9 to 27, linear-oblong, 2.5 to 3.8^{cm} in length.

T. ONOBRYCHOIDES Nutt. Rather stout for the genus, erect or nearly so: stem terete, geniculate, producing from near the summit a long-peduncled erect many-flowered raceme: leaflets oblong, silky beneath, obtuse or retuse at the apex, cuneate at the base, 2.5 to 3.8^{cm} long, a fourth as wide: flowers pale changing to red or at length purple: pods secund, finely appressed-pubescent.— Jour. Acad. Philad. **7**: 104; Torr. & Gray, Fl. **1**: 296; Engelm. & Gray, Pl. Lindh. 1: 6, 33; Gray in Hall, Pl. Tex. 7; Chapm. Fl. ed. 2, 615. *T. angustifolia* and *T. multiflora* Featherman, Bot. Rep. Louisiana Univ. **1870**: 73, acc. to Gray, Am. Jour. Sci. III., **2**: 375. *Cracca onobrychoides* Kuntze, l. c.; Vail, l. c. 29.— Dry plains, Arkansas, *Nuttall*, *Harvey*, to Louisiana, *Hale*, and Texas.

+— +— Petioles longer than the leaflets (rarely equaled by them in *T. Lindheimeri*).

++ Pods 6 to 8.5^{mm} broad: leaflets suborbicular.

T. LINDHEIMERI Gray. Soft-pubescent perennial herb with long reclining branched stems and 5–13-foliolate leaves: leaflets broadly obovate, rounded at the end, subcuneate at the base, soft-sericeous upon the lower surface; linear striate attenuate stipules very long: flowers rather numerous in erect pedunculate racemes, purple: pods velvety-tomentose.— Pl. Lindh. 2: 172; Torr. Mex. Bound. 51. *Cracca Lindheimeri* Kuntze, l.c.; Vail, 28. Sandy prairies, southwestern Texas, *Lindheimer*, *Wright*, *Palmer*, *Havard*, *Fuchs*. (Adj. Mex. where first collected by *Berlandier*.)

++ ++ Pods about 4^{mm} broad.

T. AMBIGUA Chapm. Stems prostrate or ascending, from a deep woody root, copiously pubescent to nearly glabrous: leaves 3-13-foliolate, petioles 3.8 to 6.3^{cm} long; leaflets obovate or oblong, obtuse or obtusish, 2.5 to 3.8^{cm} long, 6 to 17^{mm} broad, sparingly appressed-pubescent or glabrate above, veins red and surface paler, appressed-pubescent beneath; stipules 4 to 8.5^{mm} in length: peduncles long, ancipital, remotely 3–6-flowered: calyx very small: petals purple: pods narrow, appressed-pubescent, many-seeded.— Fl. 96; Wood, Bot. & Flor. 95. *T. hispidula* var. β Torr. & Gray, Fl. **1**: 296. *Galega ambigua* M. A. Curtis, Bost. Nat. Hist. Soc. **1**: 121. *Cracca ambigua* Kuntze, l. c. Vail, l. c. 32.— N. Carolina, *Curtis*, to Florida and Mississippi, *Pollard*; common.

Var. **gracillima**. Very slender: leaflets lance-linear, acute or acutish, seldom over 4^{mm} in breadth.— *Cracca angustissima* Vail, 32, not *T. angustissima* Rugel.— Dry pine barrens near Eau Gallie, Indian river, Florida, *A. H. Curtiss*, nos. 584, 5708; fl. July.

* * Flowers smaller; petals 6 to 8.5^{mm} long.

T. PURPUREA Pers. Slender, flexuous, branching from near the base: root long, at first filiform at length lignescent: stem erect or ascending, finely appressed-pubescent: earliest leaves 3-foliolate, the later ones 7–19-foliolate: leaflets very variable, oblong, retuse, or in var. **angustissima** (*T. angustissima* Shuttlew.) linear and acute; stipules filiform; flowers small, purple, the lowest pair usually axillary, the others forming a long slender erect raceme: pods spreading or nodding, 4^{mm} broad, appressed-pubescent under a lens, 3–5-seeded.— Syn. **2**:

329; Hook f. & Jacks. Ind. Kew. **2**: 1045. *T. leptostachya* DC Prodr. **2**: 251. 1825; Chapm. Fl. ed. 2, 616. *T. adscendens* Macfad. Fl. Jam. 257. *T. tenella* Gray, Pl. Wright. **2**: 36. *T angustissima* Shuttlew. in Chapm. l. c. 96. *Cracca purpurea* L. Spec. **2**: 752; Vail, l. c. 31. *Galega piscatoria* Ait. Kew. **3**: 71. —Sandy ridges, Florida, *Rugel*, *Curtiss*, no. 584*, *Garber*, *Simpson*. also from W. Texas, *Havard*, to Arizona. (Tropics of both hemispheres).

GRAY HERBARIUM.

BRIEFER ARTICLES.

NOTES OF TRAVEL. II.

PAYTA AND THE DESERT REGION OF PERU.

WHEN Mr. Barbour Lathrop, with whom the writer is traveling as botanical assistant, first decided to go *via* Panama to the west coast of South America, he remarked that he would show him Payta, the driest, most forsaken spot in the world. He would defy even a botanist to find so much as a single living wild plant. The donkeys of Payta are reputed, like the locusts during early days in Kansas, to eat any green plant in sight.

Payta lies less than five degrees south of the equator in the dry zone of Peru, on a coast, steadily rising from the sea in some parts, which has risen as much as forty feet within historic times. So infrequent are the rains on this coast that, when they do come, the whole native population, with crucifixes and musical instruments, goes out to welcome the river as it slowly forces its way along the bed which for seven years has been as dry as the surrounding desert. This coming of the river indicates heavy rains on the west slope of the Andes and is generally followed by showers in the region about Payta.

When we left Panama it was rumored there had been rain at Payta. Somewhat to Mr. Lathrop's disappointment, these rumors were verified when we anchored off the coast, and with the glasses discovered in one of the small valleys a green algaroba shrub (Prosopis). By looking closely we saw an almost imperceptible film of green spread over the tops of the light brownish-gray hills. On landing, we discovered that until February 9, when it rained from 10:00 P. M. until the following noon, no rain had fallen for eight years. The seventh year had failed to bring the usual periodic rains and the people were greatly alarmed lest another seven years should pass without water for the cotton crop upon which they depend largely for their support. The Payta river bed and a strip of land on each side which is overflowed form, after the subsidence of the stream, which runs only a month or two, the cultivated land of the country back of Payta. The long rooted Peruvian cotton is able to maintain itself for seven years in the dried-out river bed and yields profitable crops of the colored, short staple cotton, which is used as an adulterant for wool, occupying a place in the wool rather than the cotton market. A stroll to the top of the nearest hill at Payta showed plainly that the rain had been a heavy one, for, scattered over the nearly level table land were the hard baked remains of unmistakable mud puddles. In these, strange as it seemed to us, no plants were found, although scattered over the sand and gravel all about were young seedlings and even blooming grasses.

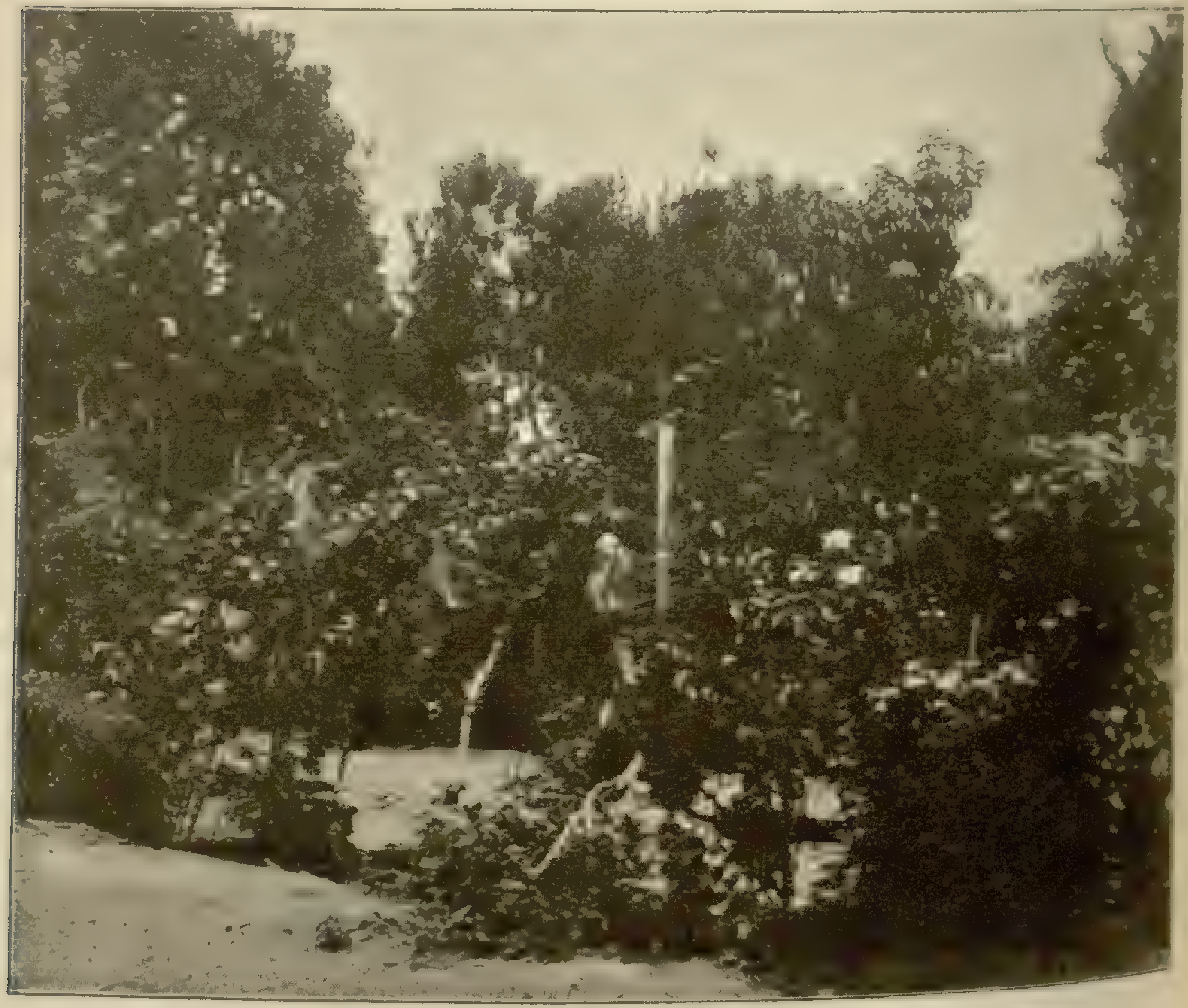

FIG. 1.—Photograph of garden at Mollendo, produced by irrigation.

The flora of Payta would not be a difficult one to write up exhaustively, provided one were on the spot at the right time. In a small pamphlet we were able to press all the phanerogamous plants which were found, without any difficulty. These plants comprise annuals whose seeds must have remained dormant since the last rain, eight years before, and perennials which have kept alive by encasing their es in thick layers of impermeable cork. There is something arkable in the ability which these desert shrubs have of reducing r transpiration surface to such a degree that they can withstand the ense insolation of this tropical region, and the even more trying ence of an extremely dry atmosphere. It is highly probable that are able, during the winter season, to absorb moisture from the which are blown in from the ocean. Owing to the cool currents

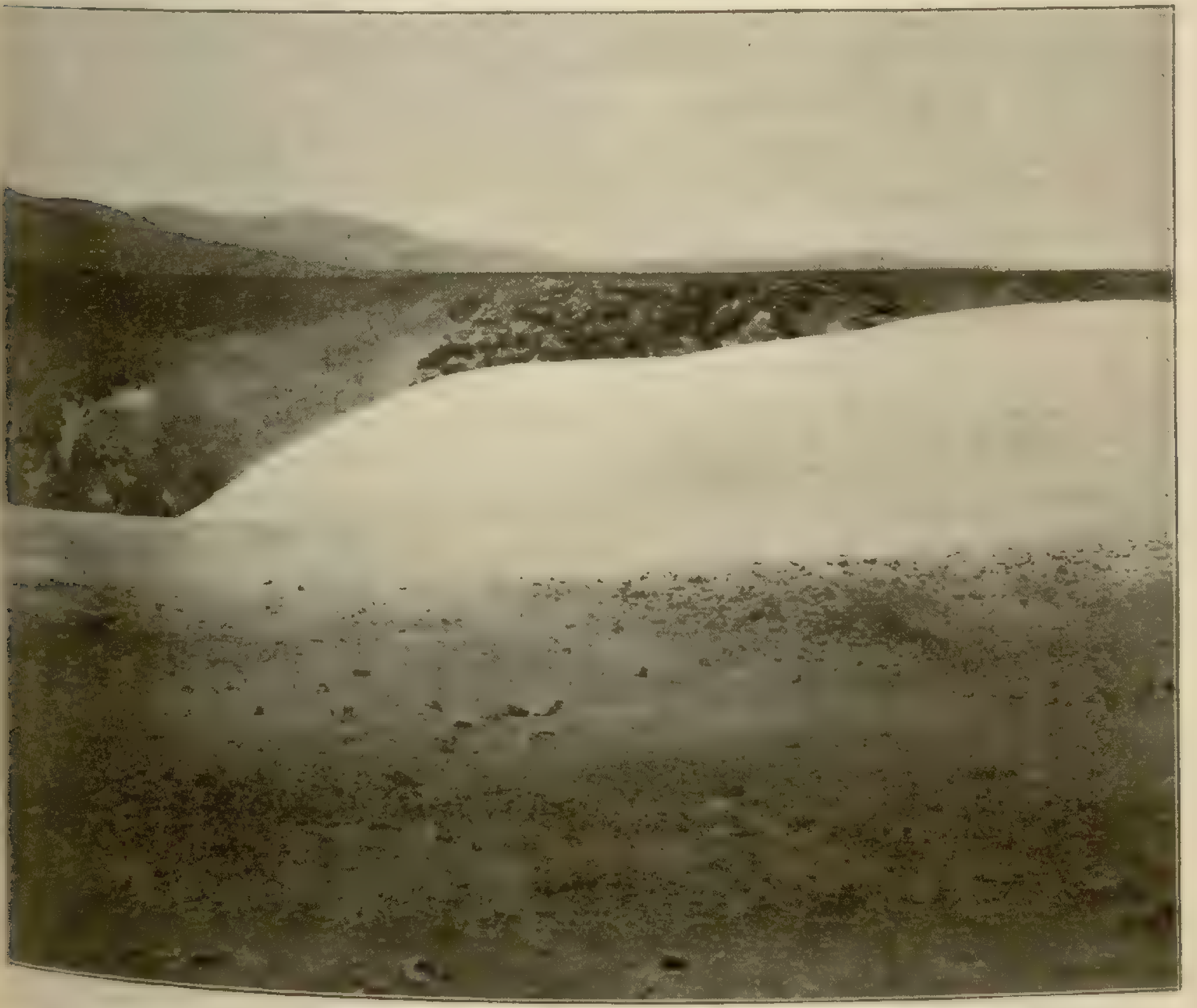

FIG. 2.—Photograph taken just outside of garden fence, showing the completely n desert.

of air which blow summer and winter across these deserts, they are not posed to such temperatures as would be expected in this latitude.

The collection of plants—the provisional flora of Payta—consists two perennials, a Prosopis and an undetermined shrub, and seven als, three grasses, a lupine, a caryophyll, a seedling amaranth, a beautiful yellow flowered oxalis. Our visit to Payta was twenty- days after the rain, and the grasses and oxalis were in full bloom. sight of hundreds of delicate yellow blossoms scattered over the Perfectly barren hillsides, and slender blades of grass so far apart that they looked like a very "bad catch" at lawn seeding, is one which few who have not been in the deserts in spring can imagine. Unlike the desert regions of our own country, with their sage brush, yuccas, and host of small tufts of grass and sedge, these deserts are for miles absolutely without a living plant. For days we steamed down the coast but aside from an occasional garden made by irrigation in the neighborhood of the towns, we saw no green plant of any description. From about Payta in Peru, to Carrizal in Chili, representing fourteen days of steamer travel. the coast presents one unbroken line of desert. At Arica it is broken by a small fertile valley, and at Carrizal the desert ends in a scanty vegetation of cacti and low growing cushion-like perennials.

At Mollendo we had an opportunity to see what this desert might have been had there been an abundance of rain. The two photographs are taken within a hundred meters of each other. One shows a private garden in Mollendo with apples, peaches, grapes, passion fruits, figs; in short a good collection of fruit and shade trees. The other is a representative view of the surrounding country as barren as a fresh lava bed.

There are below this desert at Arica, and doubtless at other points, underground sources of water, for large pepper trees which have been planted in the town square are growing as finely as they do in Gibraltar, or southern California, and overshadowing the little clubhouse at Arica is one of the largest fig trees I have ever seen.

There is no place in the world under the tropics which possesses such a cool agreeable climate as this "zona sicca," or dry zone of western South America. The contrast between the west and the east coasts of the continent at the same latitude is very remarkable. At 5 ° S. off Brazil, duck suits are necessary for comfort, while at the same latitude off Peru thin flannels are quite comfortable.

For a systematic botanist, as may be judged from the above description, there is not much of interest in this region, but from a physiological point of view it will yield some very interesting facts.

Dr. Schimper, in his *Pflanzengeographie* p. 679, calls attention to the fact that this desert region of Peru has been very little studied from an ecological standpoint. It is probable that a very small number of species will be found along this coast, and hundreds of square miles are absolutely without a living plant for years at a time. Certain localities, however, favored by the fogs, are covered in the winter season (our summer) by grasses in sufficient quantities to support numerous small herds of cattle.

The most favorable point from which to begin an ecological study of this desert region would be Payta and the inland town Piura behind it, which can be reached by railway. Having made arrangements at Piura for mules and a guide, the towns of Pacasmayo and Salaverry would not be too far apart to serve as centers for operation down the coast. The discomforts of travel through this desert, I understand, are not such as should deter any enterprising botanist from exploring it. The expenses, including steamboat travel for which the charges are twenty pounds sterling from Panama to Callao, would be easily within five dollars a day.—DAVID G. FAIRCHILD.

SECTION G (BOTANY), A. A. A. S., COLUMBUS MEETING.

THE meeting of section G began on Monday, August 21, in Townsend Hall of the Ohio State University, by a brief session for organization. In the afternoon at four o'clock, in Botanical Hall, the vice president, DR. CHARLES REID BARNES, delivered an address on *The progress and problems of plant physiology.* At the close of the address, the thanks of the section were voted to the speaker.

The reading of papers began on Tuesday, when the following were presented in full, or in abstract, or by title:

F. L. STEVENS: The fertilization of *Albugo bliti.*

FRANCIS RAMALEY: The embryo sac of *Leucocrinum montanum.*

A. S. HITCHCOCK: Notes on subterranean organs.

W. J. BEAL: Some monstrosities in spikelets of Eragostis and Setaria, with their meaning.

CHARLES E. BESSEY: Studies of vegetation of the high Nebraska plains.

A. D. SELBY: The tamarack swamp in Ohio.

WM. SAUNDERS: The breeding of apples for the northwest plains.

BYRON D. HALSTED: Field experiments with "nitragin" and other germ fertilizers.

HENRY L. BOLLEY: The duration of bacterial existence under trial environments.

Wednesday was designated *Sullivant Day*, and was used to commemorate Wm. S. Sullivant (died 1873) and Leo Lesquereux (died 1889), two most able bryologists who were long residents of Columbus. Through the initative and energy of Mrs. E. G. Britton and the assistance of a number of botanists, an exhibition of many interesting bryological books and pamphlets, type specimens and original drawings of mosses, photographs, portraits, and autographs of bryologists, maps of distribution, etc., was given during the day in the large lecture room in Botanical Hall, where Wednesday's sessions were held. Portraits of Sullivant and Lesquereux, loaned by their daughters, formed the center of interest. This exhibition attracted much attention and was warmly commended.

The exercises in honor of Sullivant and Lesquereux were as follows:

Professor W. A. Kellerman read a portion of Gray's tribute to Sullivant.

Professor C. R. Barnes read a biography of Lesquereux.

Mrs. E. G. Britton gave a brief account of the species of mosses named for Sullivant.

A letter was read from Professor Arthur Hollick regarding the paleobotanical work of Lesquereux.

Professor L. M. Underwood gave a brief outline of the progress in the study of the Hepaticæ of North America, and Mrs. Britton for the Musci. Both addresses were illustrated by the books, pamphlets, photographs and maps of the exhibition.

Professor F. E. Lloyd exhibited the plates and type specimens of the twelve new species of liverworts described by Dr. M. A. Howe in his recent monograph.

Professor W. A. Kellerman presented to each member of the section a set of six species associated with the names of Sullivant and Riddell, an early Ohio botanist. The specimens were from type localities in most cases. They were *Sullivantia Sullivantii*, *Lonicera Sullivantii*, *Arabis patens*, *Solidago Ohionis*, *S. Riddellii*, and *Trillium nivale.*

Mrs. E. G. Britton also distributed specimens of *Orthotrichum Ohioense* and *Bryoxiphium Norvegicum.*

The following papers were also read:

CHARLES MOHR: Notes on the moss flora of Alabama.

A. J. GROUT: Suggestions for a more satisfactory classification of the pleurocarpous mosses.

BRUCE FINK: Notes concerning the study of lichen distribution in the Mississippi valley.

W. C. STEVENS: Botanical teaching in the secondary schools.

IDA CLENDENIN: Botanical teaching in the secondary schools.

The two papers on botanical teaching had been prepared by request and evoked an interesting discussion.

On Thursday the following papers were read:

H. A. HARDING: On the occurrence of the black rot of cabbage in Europe.

CHARLES E. BESSEY: One thousand miles for a fern.

WALTER T. SWINGLE: A summary of our knowledge of the fig.

WM. TRELEASE: The classification of botanical publications.

As a result of the discussion of this paper, transmitted by the Botanical Society of America, the following resolution was adopted:

Resolved, That Section G recommends as a basis for the classification of a botanical library the decimal system now in common use in the United States. The section requests that the suggestions embodied in Dr. Trelease's scheme for classification be adapted, so far as possible, to that system, and that the paper be then published in *Science* for the purpose of eliciting discussion.

EDWIN B. COPELAND: The geotropism of the hypocotyl of cucurbits.

A. F. WOODS: The destruction of chlorophyll by oxidizing enzymes.

Apropos of this paper the following action was taken:

Resolved, That Section G express its gratification at the appointment of an eminent physiological chemist to the staff of the Division of Vegetable Physiology and Pathology of the Department of Agriculture.

The secretary was instructed to communicate this resolution to the Secretary of Agriculture.

C. O. TOWNSEND: The effect of hydrocyanic acid gas upon the germination of seeds.

W. G. JOHNSON: Some physiological effects of hydrocyanic acid gas upon plants.

A communication was read from the Cambridge Botanical Supply Co., which has been publishing the card edition of the bibliography of American botany under the direction of the Section's Committee on Bibliography, stating that the publishers must terminate the present arrangement at the close of 1899 on account of the fact that the number of subscribers was insufficient to meet the cost of publication. After discussion the committee was increased to five by the appointment of D. T. MacDougal, of New York Botanical Garden, and J. F. Cowell, director of the Buffalo Botanical Garden. The section expressed its approval of the card index and empowered the committee to arrange for its continuance in any feasible way.

On Friday the following papers were read:

J. C. Arthur: The cultures of Uredineæ in 1899.

Francis E. Lloyd: The embryology of *Vaillantia hispida.*

A. D. Selby: The flora of Franklin county, Ohio.

Erwin F. Smith: The fungous infestations of agricultural soils in the United States.

C. E. Bessey: Are the trees advancing or retreating upon the Nebraska plains?

Wm. Saunders: Useful trees and shrubs for the northwest plains of Canada.

H. L. Bolley and **L. R. Waldron**: The occurrence of calcium oxalate and lignin during the differentiation of the buds of *Prunus Americana.*

Hermann von Schrenk: Two diseases of Juniperus.

Wm. B. Stewart: Etiolative reactions of Sarracenia and Oxalis.

Julia B. Clifford: The mycorhiza of Tipularia.

Henry Kraemer: The crystals in *Datura Stramonium.*

At 3 P.M. on Friday the section adjourned, *sine die.*—C. R. B.

BOTANICAL SOCIETY OF AMERICA.

The fifth annual meeting was held in Columbus, Ohio, August 18-19, under the presidency of Dr. Lucien Marcus Underwood. All sessions were held in buildings of the Ohio State University. The address of the retiring president, Dr. Nathaniel Lord Britton, upon *The development of the New York Botanical Garden* was given in the chapel of University Hall on Friday evening. It was copiously illustrated with lantern slides, showing the original site, with its natural beauties, the progress of the planting, buildings, etc. The address will be published in full in a later number of the Gazette. At the close of the address the thanks of the audience were voted to the speaker for the interesting and able presentation of facts regarding the great institution of which he is director. The following papers were read in full or in abstract or by title before the society. Abstracts of some of them will be found in later pages:

Charles E. Bessey: *Apetaly and diœciousness.*

Bradley M. Davis: *The spore-mother-cells of Anthoceros.*

Daniel T. MacDougal: *Symbiosis and saprophytism.*

David M. Mottier: *The effect of centrifugal force upon the cell.*

Nathaniel L. Britton: *The American species of Arisæma.*

JOSEPH C. ARTHUR: *The Uredineæ occurring upon Phragmites, Spartina, and Arundinaria in America.*

BYRON D. HALSTED: *Distribution of American Erysipheæ.*

BRADLEY M. DAVIS: *Gametes and gametangia of the Phycomycetes.*

WILLIAM TRELEASE: *Classification of botanical publications.* The society requested that this paper be read also before section G, A. A. A. S.

DANIEL T. MACDOUGAL: *Etiolative reactions.*

LUCIEN M. UNDERWOOD: *The foundations of genera in ferns.*

The officers elected for 1900 are: BENJAMIN LINCOLN ROBINSON, *president;* BYRON DAVID HALSTED, *vice president;* ARTHUR HOLLICK, *treasurer;* GEORGE FRANCIS ATKINSON, *secretary;* B. T. GALLOWAY and D. P. PENHALLOW, *councillors.*

The new members elected were: J. M. Macoun, Geological Survey, Ottawa, Can.; W. J. Beal, Agricultural College, Mich.; C. F. Millspaugh, Field Columbian Museum, Chicago; Marshall A. Howe, Columbia University, New York.

Amendments to the constitution were adopted, creating as additional classes of members, life members, associates, and patrons. The new sections are as follows:

Section 3. Life Members.—Any member of the Society may become a Life Member by the payment to the treasurer of one hundred dollars at any one time. Life membership fees shall be added to the permanent fund of the Society.

Section 4. Associates.—Associates of the Society may be elected in the manner already prescribed for members. Before the first of January following his election, each Associate shall pay into the treasury of the Society annual dues to the amount of five dollars. Associates shall have all the privileges of members except that of voting, and of holding office. Subsequent to the adoption of this provision members shall be chosen only from the list of associates.

Section 5. Patrons.—The payment to the treasurer of a sum not less than two hundred and fifty dollars at any one time, or a bequest of such sum, shall constitute the donor a Patron of the Society. The names of Patrons shall be published with the annual lists of officers and members, and the Patrons shall be entitled to receive copies of all the publications of the Society. Patrons' fees shall be added to the permanent fund of the Society.

The report of the treasurer shows funds on hand amounting to $1438.27, of which $1260 is on deposit in savings banks. A committee was appointed to invest funds where a higher rate of interest may be obtained than from savings banks. The total assets of the Society are now $1517.59.—C. R. B.

BOTANICAL CLUB, A. A. A. S.

UNDER the presidency of Dr. Byron B. Halsted, with Professor A. D. Selby acting as secretary, the Botanical Club listened to the following brief communications:

C. E. BESSEY: A greasewood compass plant.

C. E. BESSEY: A visit to the original station of the Rydberg cottonwood.

N. L. BRITTON: Report on Mr. Heller's botanical exploration of Porto Rico.

F. S. EARLE: Tomato fruit rot.

W. J. BEAL: The botanical club of the Michigan Agricultural College.

WM. SAUNDERS: The arboretum and botanic garden of the Central Experimental Farms, Ottawa, Canada.

F. E. LLOYD: On two hitherto confused species of Lycopodium.

L. M. UNDERWOOD: What shall we regard as generic types?

L. C. CORBETT: A device for registering plant growth.

A. D. SELBY: The introduced species of Lactuca in Ohio.

O. F. COOK: Notes on some of the work of the Division of Botany of the U. S. Department of Agriculture.

THOMAS A. WILLIAMS: Some features of the investigations on grasses and forage plants, in charge of the Division of Agrostology, U. S. Department of Agriculture.

J. W. T. DUVEL: A brief embryological study of *Lactuca Scariola* L.

N. L. BRITTON: Notes on the northern species of Celtis.

N. L. BRITTON: Remarks on some species of Quercus.

W. J. BEAL: The introduction of *Cabomba Caroliniana* in Michigan.

C. E. BESSEY: The wilting of *Cleome integrifolia*.

W. A. KELLERMAN: Labels for living plants.

H. L. BOLLEY: The position of the fungi in the plant system as indicated by the work on the organisms of nitrification.

L. M. UNDERWOOD: Summary of our knowledge of the distribution of fungi in America.

C. E. BESSEY: The powdery mildew of *Polygonum aviculare*.

A. D. SELBY: On *Plasmopara Cubensis*.

A. S. HITCHCOCK: Distribution of some Kansas plants.

W. R. LAZENBY: Unusual development of leaves and growth of plants from cuttings.

A. D. HOPKINS: Some botanical notes by an entomologist.

A. S. HITCHCOCK: Some wheat crosses.

On Thursday morning the Club adjourned *sine die*.

THE SEXUALITY OF THE TILOPTERIDACEÆ.[1]

Ectocarpus pusillus Griffiths or *Acinetospora pusilla* Bornet possesses two forms of reproductive organs, the unilocular and plurilocular sporangia, whose elements present conditions intermediate between zoospores and aplanospores. I have recently encountered a third form of reproductive organ, the monosporangium, identical with that of *Haplospora Vidovichii* Bornet or *Heterospora Vidovichii* Kuckuck, and this last species ought henceforth to be placed in the genus Acinetospora.

These monospores are uninucleate, like the so-called oospheres of *Scaphospora speciosa* (said to be the sexual form of *Haplospora globosa*), and are covered by a membrane derived from the interior of the monosporangium, as are likewise the 4-nucleate monospores of *Haplospora globosa*. Since they possess a membrane before their dehiscence they cannot be fertilized. On account of the frothy structure of their protoplasm and the great variation in their dimensions, they resemble neither oospheres nor spores. They germinate readily in cultures and develop little plants that bear the same kind of reproductive organs. I regard them as *gemmae* or *propagula*, and the organ that contains them is a pseudosporangium. From what we know of the Cutleriaceæ and Ectocarpus it is possible that the plurilocular sporangia of *Acinetospora pusilla* are female organs whose oospheres germinate parthenogenetically. The antheridia will undoubtedly be found some day.

The same conclusion as to the nature of the monospores is justified in Tilopteris and Haplospora. We are no longer able to admit that these plants possess spores and oospheres similarly situated, of like form and similar dimensions, with the same protoplasmic structure and identical methods of germination, but that the spores germinate normally while the oospheres develop parthenogenetically. It seems more satisfactory to say that one or the other of the elements are propagula and that the 4-nucleate propagula are those that have begun to germinate upon the mother plant.

We shall say then that according to the present state of our knowledge the early known representatives of the Tilopteridaceæ, Tilopteris, and Haplospora propagate themselves solely vegetatively. The antheridia of Tilopteris give rise to true antherozoids that really seem to be rudimentary structures, but it is by no means certain that they are the same as those organs of Haplospora, however similar the type. We do not know of female organs, but we may foresee that they would have the form of plurilocular sporangia with a median cavity and a separate dehiscence for each little cell. Similarly one may foresee that the antheridia of Acinetospora will be provided with a median cavity and that the dehiscence will be common and terminal.

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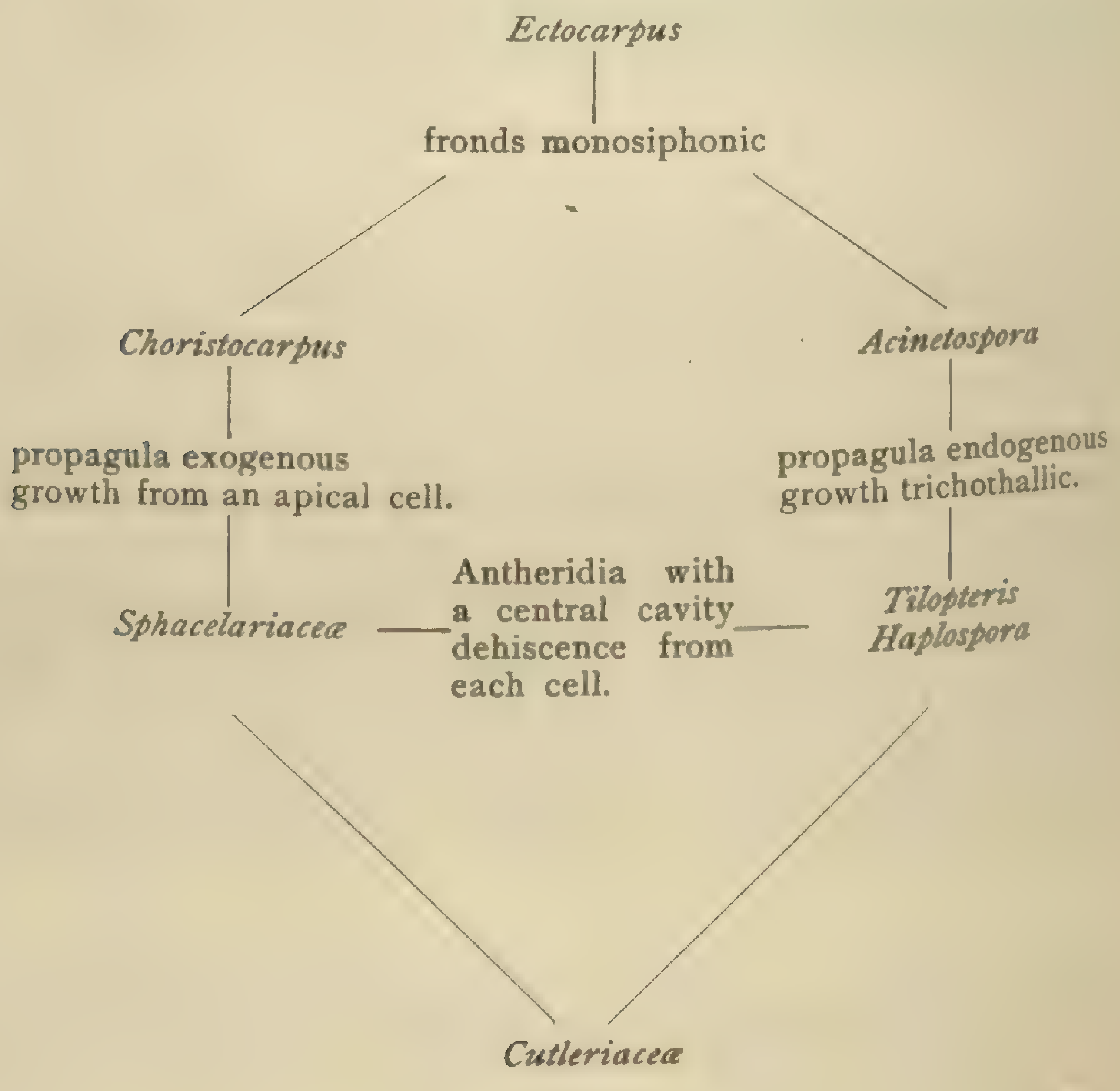
[1] This contribution is a *résumé* by the author of the paper entitled "Les Acinetospora et la sexualité des Tilopteridacées," Jour. de Bot. **13**: —. 1899. Translated by Dr. Bradley M. Davis.

The Tilopteridaceæ have no affinity with the Fucaceæ with which they have often been placed in the classical works. On the contrary they approach very closely the Ectocarpaceæ and Cutleriaceæ. They may be divided into two tribes, the ACINETOSPOREÆ (genus Acinetospora), more closely related to the first; and the HAPLOSPOREÆ (genera Tilopteris and Haplospora), more closely related to the second. When these plants shall show us organs that are as yet unknown we shall without doubt have to raise the preceding tribes to the rank of families, the Acinetosporaceæ and Haplosporaceæ. I have shown in the table below a way in which the affinities of these plants may be represented.—CAMILLE SAUVAGEAU, *Dijon, France.*


Ectocarpus
fronds monosiphonic
Choristocarpus
Acinetospora
propagula exogenous
growth from an apical cell.
propagula endogenous
growth trichothallic.
Antheridia with
a central cavity
dehiscence from
each cell.
Sphacelariaceæ
Tilopteris
Haplospora
Cutleriaceæ

FLOWER VISITS OF OLIGOTROPIC BEES.

Since the proof of my last paper in this journal was read I have had occasion to doubt the correctness of the statements regarding Epeolus, on pages 35–37, and have asked Mr. Ashmead if he still held the views credited to him. In a letter of August 2, he writes that the nests were evidently made by *Entechnia taurea* and that the Epeolus was merely entering them, not making them, as he supposed at the time. Epeolus, therefore, comes under the same category as Nomada. After all, the phenological position of the genus corresponds pretty well with that of Melissodes upon which most of the species are probably inquiline.

In the table of oligotropic bees *Xenoglossa cucurbitarum* should be included. Lately I have found it collecting pollen of *Cucurbita pepo*. It also visits *Citrullus vulgaris*, *Asclepias Cornuti*, *Ipomœa nil* and *I. pandurata*. It has been taken at Ames, Iowa, by Miss Alice M. Beach, on flowers of "summer squash;" at Mesilla, New Mexico, by Mr. Cockerell, on flowers of *Cucurbita perennis*; at Metropolis, Ill., by Mr. Hart, ou *Martynia proboscidea*, and is mentioned in the Gazette (17:65) under the MS. name *X. brevicornis*. I suspect that all of our species of Xenoglossa get their pollen exclusively from Cucurbitaceæ.—Charles Robertson, *Carlinville, Ills.*

QUERCUS ELLIPSOIDALIS IN IOWA.

Mr. William D. Barnes, of Morgan Park, Illinois, has placed in my hands specimens of an undetermined oak, collected by him in 1895 at Big Rock, Scott county, Iowa, which proves to be *Q. ellipsoidalis* Hill. Mr. Barnes and two collaborators are preparing a catalogue of the plants of Scott and Muscatine counties for the Davenport Academy of Sciences, and have been unable to determine the name of this oak. The field note accompanying this specimen reads: "Tree with smooth bark, and with the general aspect of *Q. palustris*." It is a fruiting branch, the leaves quite small and rather narrower than those commonly found near Chicago, but such as may frequently be seen on individual branches, or as to size may characterize nearly an entire tree. The acorn is one of the smaller kind, closely resembling the one figured in *Plate II, e*, Botanical Gazette, March 1899.—E. J. Hill, *Chicago*.

A NEWLY OBSERVED STATION FOR *GALINSOGA HISPIDA*.

SOME time ago the writer[2] called attention to the fact that besides *Galinsoga parviflora* Cav. and its var. *hispida* DC., another quite distinct species, *G. hispida* Benth., had been found in the Atlantic states, occurring at Camden, New Jersey, where it was collected on waste lands by Mr. C. F. Parker. This species was again found last October by Mr. Howard Schriver at several points in Cumberland, Maryland. Mr. Schriver, noticing its differences from *G. parviflora* Cav., sent specimens to the U. S. National Museum, whence they were kindly forwarded to the writer by Dr. J. N. Rose.

Mr. Schriver reports the plant as not only forming a large mass of vegetation (5 or 6 meters long) near a "bonded warehouse" or sort of cattle depot, but also extending down the banks of an adjacent stream where individuals were scattered on steep cliffy slopes of Helderberg limestone, even reaching the water's edge. A sporadic specimen was also found on a country road not far away. While the presence in the neighborhood of the cattle yard, a railway, and a distillery would readily account for the introduction of plants from a distance, the present species in the luxuriance of its growth and tendency to spread into the indigenous vegetation would suggest that it has found congenial conditions and is likely to persist. The species is readily distinguished by its "pink" or "red" rays (drying purple) and by it short pappus, which is about half the length of the achene. In foliage and rather copious spreading pubescence it resembles *G. parviflora*, var. *hispida* DC., but both *G. parviflora* and its variety have white rays and much longer pappus (nearly or quite the length of the achene).

As *G. hispida* Benth. is likely to be found at other stations in the middle Atlantic states it may be worth while to cite its more important synomymy, which is as follows:

Vargasia caracasana DC. Prodr. **5**: 676. 1836.

Galinsoga hispida Benth. Bot. Sulph. 119. 1844.

G. brachystephana Regel, Ind. Sem. Hort. Turic. 2. 1846.

G. caracasana Sch. Bip. Linnaea **34**: 529; also Bull. Soc. Bot. Fr. **18**: 86. 1865.—B. L. ROBINSON, *Gray Herbarium.*

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[2] Proc. Am. Acad. **29**: 326.

CURRENT LITERATURE.

BOOK REVIEWS.

Engler and Prantl's Pflanzenfamilien.

This great work has been noticed from time to time in the BOTANICAL as the various parts have appeared. But now that Volumes II–IV plete, which contain the siphonogams, the time seems appropriate more extended notice. The first part appeared in 1887, and twelve later the three volumes of siphonogams were finished. The publication three volumes of Bentham and Hooker's *Genera Plantarum*, covering ground, but with no such breadth of treatment, extended from 1883, a period of twenty-one years. There is no definite statement as completion of Volume I, devoted to cryptogams, but several sections have been published, and other parts are appearing with reasonable

So far as statistics are concerned, it may be of interest to note that, excluding the indexes and the cryptogams, there are twenty-six sections of the work each with its separate index, and forming handy laboratory volumes. number of genera treated by the fifty-seven collaborators is 8218. The are 6097 in number, the original illustrations 3026 (woodcuts 3023, gravures 3), and the individual figures 19,366. The total price is *M* 436, bound in eleven half morocco volumes *M* 474.50.

When one considers such details he is impressed by the magnitude of the and still more by the organizing power which has kept the large plans ration through so many years. The editorial work must have been to bring contributions necessarily heterogeneous into a reasonable of uniformity. But while the details are impressive they do not the importance of the work. That it marks an epoch in taxonomic does not need to be stated as a prophecy, for during its publication it has achieved this distinction. Lists, manuals, and herbaria were the Engler and Prantl sequence long before the work was complete. was due to the fact that it sought to relate plant groups upon the basis s known concerning them, and discarded the old groupings which been the laughing stock of biologists. In other words, this great breathes into taxonomy the modern biological spirit, and makes it more a set of names.

rior to all previous general works in its spirit, it is alone in the and beauty of its illustrations. Every family is thoroughly and admirably illustrated, and we venture the prediction that many of these figures will become classic in future texts. No such collection of figures representing the plant kingdom exists, and they give a conception of plants in general that can be obtained from no other publication. The figures and text include not merely those structures which may be said to have taxonomic importance, but anatomical peculiarities of each family are set forth. All through the work the ecological standpoint is prominent, and the sections on geographic distribution are among the most valuable.

It is to be expected that the treatment is unequal, and the different parts of very different degrees of merit, but with fifty-seven collaborators this cannot be avoided. It seems to most botanists far more important to complete a work within a reasonable time, and so establish a usable datum-line, than to drag it out indefinitely and allow one part to be out of date before another is published. In general the treatment will be regarded as conservative, there being apparent no desire for change if existing lines can be used at all. In so delicate a matter as nomenclature, as is well known, the "Berlin rules," which are in fact the Engler rules, are drawn up in the spirit of compromise, not going to either extreme, and probably satisfying neither set of extremists. No set of rules proposed, however, has had as yet such a tremendous advantage of general usage as this great work will compel for the Berlin rules.

It is impossible to mention in detail the views advanced as to the evolution of plant groups. There will be much difference of opinion as to minor points, for many smaller groups, through lack of adequate investigation, had to be "lumped," but in the judgment of the reviewer the main lines of evolution suggested will stand, which are in brief as follows: spiral arrangement and indefinite numbers to cyclic arrangement and definite numbers; naked flowers to differentiation of calyx and corolla; apocarpy to syncarpy; polypetaly to sympetaly; hypogyny to epigyny; actinomorphy to zygomorphy. That cases of "reduced flowers" occur there can be no doubt, but that the great majority of so-called cases of reduction are really primitive in character seems hardly less doubtful.—J. M. C.

Ferments and fermentation.

THE attention which the various problems connected with fermentation have received during the past decade and the interest, both theoretical and practical, which attaches to the investigation of these problems make doubly welcome a book on the soluble ferments from the hand of Professor Reynolds Green.[1] In it he has sought to bring together, so far as possible, the results already reached, and to indicate the view of the processes fermentation to which these results point. For it can hardly be said that the results now attained furnish any adequate explanation of fermentation; though they remove it more completely from the realm of so-called "vital" action, and refer it to the category of equally inexplicable catalytic phenomena.

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[1] GREEN, J. REYNOLDS: The soluble ferments and fermentation. 8vo. pp. xii + 480. Cambridge: The University Press. 1899. 12 s. [New York: The Macmillan Company.]

After discussing the nature of fermentation and its relation to enzymes, the author gives a detailed account of diastase (60 pp.), inulase, cytase, sugar-splitting enzymes, glucoside-splitting enzymes, proteolytic enzymes (57 pp.), fat-splitting enzymes, clotting enzymes (48 pp.), ammoniacal fermentation, oxidases, alcoholic fermentation, and the fermentative power of protoplasm. The work closes with chapters on the secretion, constitution, and mode of action of enzymes. Of course the treatment of these topics includes a discussion of the discovery, occurrence, preparation, and behavior of enzymes in both animal and plant bodies.

A work like this is not open to adverse criticism. It is rather to be commended without stint. Indeed, every physiologist will be thankful to know and to have at hand this compact but full summary of researches, accompanied as it is, by an extensive bibliography, leading to further details in the original papers. One notices with pleasure that even the results contradictory to the general trend of investigation are clearly stated. This engenders confidence in the fairness of the work; a confidence which the closest scrutiny justifies.

The bibliography, which the author modestly says is not exhaustive, is especially to be commended. Its extent will be appreciated when it is stated that it contains about 800 titles! The citations would have been improved had some uniform system been followed. Here is an assortment of five styles taken from p. 449: (1) Zeit. f. Biologie Bd. x. 92; (2) Zeitsch. f. klin. Med. [illegible]. 231; (3) Cent. f. Bact. 1891. Bd. 10. 401; (4) Cent. f. Bakt. 15 (1894). 722; (5) Ber. d. deut. chem. Gesell. 23 (1890). 3689; (6) Ber. d. deut. chem. Gesell. (1895), 1433. This will be looked upon by many as of small consequence; and so it is, in comparison with the value of the work. But the defect is so unnecessary and so easily avoided that it seems the greater pity that it should mar so good a bibliography. Moreover, calling attention to it here may serve to emphasize a point which many scientific writers sorely need to lay to heart.

Users of this work will be thankful that the editor of the *Cambridge Natural Science Manuals*, of which this is one, had an index prepared, in spite of the author's insistence that one was unnecessary. They will only be sorry that the index is not fuller, and that it does not include the names of authors, for which the bibliography must be consulted.—C. R B

NOTES FOR STUDENTS.

BEFORE THE Botanical Society of America, at the Columbus meeting, Professor D. T. MacDougal read a paper on *Symbiosis and saprophytism*, of which the following is a synopsis :

At the last meeting of the society I read a paper in description of my work upon a large number of herbaceous mycorhizal plants,[2] and a short note was presented before the meeting of the Society for Plant Physiology and Morphology, in December 1898, in which a delimitation of the terms saprophytism and symbiosis was attempted. Attention was also called to the fact that only two seed plants, Wullschlægelia and Cephalanthera, may be truly designated as saprophytes, all other species of so-called holosaprophytes being symbiotic with mycorhizal fungi.

During the last year my efforts have been directed first to ascertain the adaptations undergone by these true saprophytes, and compare such changes with those undergone by mycorhizal forms. Secondly, evidence which might have a bearing upon the physiological relations of a seed plant and its mycorhizal fungus has been carefully sought for.

Cephalanthera, the saprophyte examined, showed alterations in structure generally similar to those of mycorhizal forms ; but, in exception to the majority of chlorophylless species, it retains the stomata of the leaves. and has developed no underground transpiratory organs.

The roots, unlike those of most mycorhizal forms, are deeply buried in the soil, on account of which the number of good herbarium specimens to be found is extremely small. Two types of these organs are present : a fibrous one with a reduction and fusion of the stelar components, and with radially elongated cortex. This variation has hitherto been regarded as a result of the presence of a fungus as in mycorhiza, while as a matter of fact it is an adaptation to humus foods. The second type of root is devoted to storage and has a normal multiplication of the cortical elements. Both types agree closely with the two kinds of roots formed by Wullschlægelia, which still further strengthens the conclusions given above.

The lack of apparatus for the conversion of radiant energy indicates that these two saprophytes must receive their entire supply from the chemical energy of the humus compounds taken up, and we may decide inferentially that substantial modifications of the minute mechanism of absorption must have taken place, entailing also unusual osmotic conditions. In general these two seed saprophytes live like fungi, although capable of forming starch.

The coralloid formations on the offsets of Calypso, which are found from Norway to Washington, have been examined, and the result of the development of these adventitious mycorhizas is quite similar to that already described in Aplectrum. The occurrence of the coralloid mycorhiza is coupled

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[2] Published in the *Annals of Botany* 13 : —. March 1899.

[illegible] a diminution of the leaves, the roots, and the storage organs, indicating increased acquisition of highly organized food-material; a variation which [illegible] have the same ultimate influence on the species as in Aplectrum, and is doubtless responsible for many of the so-called aberrant forms of leaves and flowers reported.

This species and *Corallorhiza Arizonica* were examined with especial reference to the relations of the seed plant and the endotropic fungus. *Corallorhiza Arizonica* is entirely free from chlorophyll, and the aerial shoot has lost the stomata. The subterranean rhizome and its coralloid branches are each furnished with separate types of motile stomata, however, which are open during the entire year, but clearly serve transpiratory and respiratory needs alone.

The arrangement of the fungus is one which has been found in nearly all of the endotropic mycorhizas hitherto examined, and its study has yielded some important results.

The hyphæ of the fungus extend themselves in comparatively straight lines longitudinally toward the tip of the organ in the sub-epidermal region of the cortex, and this portion remains alive even in old members constituting what may be designated as the "vegetative mycelium." The entrance of the [illegible] of the vegetative mycelium into the young cortical cells causes almost no disturbance in the character of the latter, and no important interchange between the two plants ensues in this region.

As the growth of the root and the contained vegetative mycelium goes on, the younger portion of the mycelium sends out hyphal branches which extend through the epidermis and may or may not traverse the hairs into the humus soil; these are very clearly absorbing organs. At the same time another set of branches penetrate to the median region of the cortex and both [illegible] and undergo profound disturbances. The tips of the hyphæ are [illegible] to the vicinity of the nuclei. Dense coils and sometimes large [illegible] are formed which serve as organs of interchange between the fungus and the seed plant. The starch contained in the cortical cells inhabited by these organs is used by the fungus; then, with the maturity of this general [illegible] the organs of interchange and the large amount of contained proteid are set free and become available to the higher plant.

Here then is the character of the partnership of the two plants: The seed plant furnishes a habitat for the vegetative mycelium of the fungus and yields to it certain carbohydrate foods, principally starch. The fungus takes up the humus products from the soil by means of its external branches, conducts them to the inner branches in the cortex of the higher plant, and manufactures proteids, of which a portion is used in its own metabolism, of course, but the greater part is yielded to the seed plant.

It follows as a necessary corollary from the above conclusions, that Frank's [illegible] that mycorhizal adaptations are fungus traps and that the seed plant derives the entire advantage from the association is no longer tenable. Likewise the theory of Janse, that endotropic fungi which form mycorhizas are negatively chemotropic to oxygen and bear the same relation to the seed plant as the organism in the leguminous tubercle, is not capable of universal application. Such relation has been proven by Nobbe and Hiltner between Podocarpus and the peronosporous fungus which forms endotropic mycorhiza with it, but in no other instance.

At this stage of our investigations, then, two distinct physiological types of endotropic mycorhizas are recognized: One adapted for nitrogen fixation and a second for the absorption and modification, perhaps oxidation, of humus products by the fungus and their liberation in the tissues of the higher plant. The greater number of examples are included under the last type.

Ectotropic or sheathing mycorhizas such as Monotropa perhaps approximate more nearly to the latter type.[3]

THE PAPER read before the Botanical Society of America, at Columbus, entitled "The effect of centrifugal force upon the cell," by Professor D. M. Mottier, discussed in detail the effect of centrifugal force varying from [illegible] to 1900 times that of gravity, acting for definite short periods of time, upon cells of certain algæ, mosses, and phanerogams. In all cells operated with the movable plasmic contents, together with the inclusions, were made to fall into a compact mass at the end of the cell. In cells which were not killed outright or too badly injured, so that death resulted soon afterwards, the displaced cell-contents gradually redistributed themselves in due course of time.

The most strikingly interesting phenomena are presented by dividing cells and in the behavior of the nucleolus. In dividing cells of such algæ as Cladophora and Spirogyra, the cell-wall in process of formation at the time of centrifugal action was never completed. The chloroplasts, pyrenoids and other displaced contents, on becoming redistributed, pass back through the opening in the partly formed cell wall provided this opening were not too small.

Cells often divide normally before the contents become redistributed, especially in Cladophora and Spirogyra, thus giving rise to two daughter cells of unequal size, a smaller one appearing a deep green from the large amount of chlorophyll, and a larger one partly colorless with only a little chlorophyll. In certain cells of the plerome of *Zea Mays* and other phanerogams, the nucleolus was thrown out through the nuclear membrane into the cytoplasm. These nucleoli never re-entered the nucleus.

Other important observations were presented touching upon the dividing cell and nucleus.[4]

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[3] The full paper will be published in the *Bulletin of the Torrey Botanical Club.*

[4] This paper is published in the Annals of Botany for September 1899.

NEWS.

Dr. E. B. Copeland of the State Normal School, Chico, California, has been appointed instructor in botany in the University of West Virginia.

Dr. Henry C. Cowles, of the University of Chicago, has spent several weeks with a party of advanced students at Marquette, Mich., prosecuting ecological studies on the adjacent flora.

Professor P. H. Rolfs, formerly of Lake City, Florida, has accepted a position at Clemson College and Experiment Station. His post office is Clemson College, S. C.

The Smithsonian Institution is soon to appoint an assistant in cryptogamic botany, with a salary of $75 a month. The civil service examination will be held November 5 and 6.

We learn from *Science* that Aven Nelson, botanist of the Wyoming Experiment Station, is making this summer an extended survey of Yellowstone National Park and adjacent forest reserves.

The officers-elect of the Botanical Club of the A. A. A. S. are Professor F. S. Earle, of Auburn, Ala., *president;* Professor A. D. Selby, of Wooster, O., *vice president;* and Professor F. E. Lloyd, of New York, *secretary.*

Mr. George T. Moore, who has been an assistant in the cryptogamic laboratory at Harvard University, has accepted an instructorship in Dartmouth College, in charge of botany. This appointment recognizes botany in Dartmouth as a sister department in zoology.

This summer has witnessed the establishment of another inland biological station. This one is at Winona lake, under the direction of Professor Eigenmann, with Professor D. M. Mottier in charge of botany. It is closely associated with Indiana University.

The Department of Botany of the Marine Biological Laboratory has had a very successful session. Dr. Davis and Mr. Moore, of the staff, Dr. [illegible] and Professor Cook spent the summer at Woods Hole, and Professors Campbell, Atkinson, MacFarlane, Mottier, Penhallow, Kraemer, and Drs. MacDougal, Shaw, Townsend, and Smith made shorter visits to the laboratory. Never before have so many botanists been present during a session.

"MOULDS, MILDEWS, AND MUSHROOMS," is the alliterative title of a guide to the systematic study of the fungi and mycetozoa and their literature, by Professor Lucien Marcus Underwood of Columbia, author of *Our Native Ferns and their Allies*. It is to be issued shortly by Messrs. Henry Holt & Company.

DR. WALTER S. SWINGLE has returned to this country after several months of travel abroad. He has brought with him many interesting and valuable economic plants that will be given an opportunity to make this country their home. The date palm and the truffle are, perhaps, the best known forms whose introduction into this country would be greatly welcomed.

THE SECRETARY of Agriculture has planned "a publication which shall contain a résumé of the achievements of the United States in every branch of science as related to agriculture during the nineteenth century, for distribution at the Paris Exposition." Each of the bureaus and divisions charged with scientific work has been directed to contribute one or more articles reviewing the application to agriculture of the science with which it is concerned. The division of forestry intends to contribute a short history of forestry in the United States and also an account of the efforts of private land owners to apply the principles of forestry. The division solicits correspondence regarding every such effort in order that it may make a proper showing of the extent of this sort of work.

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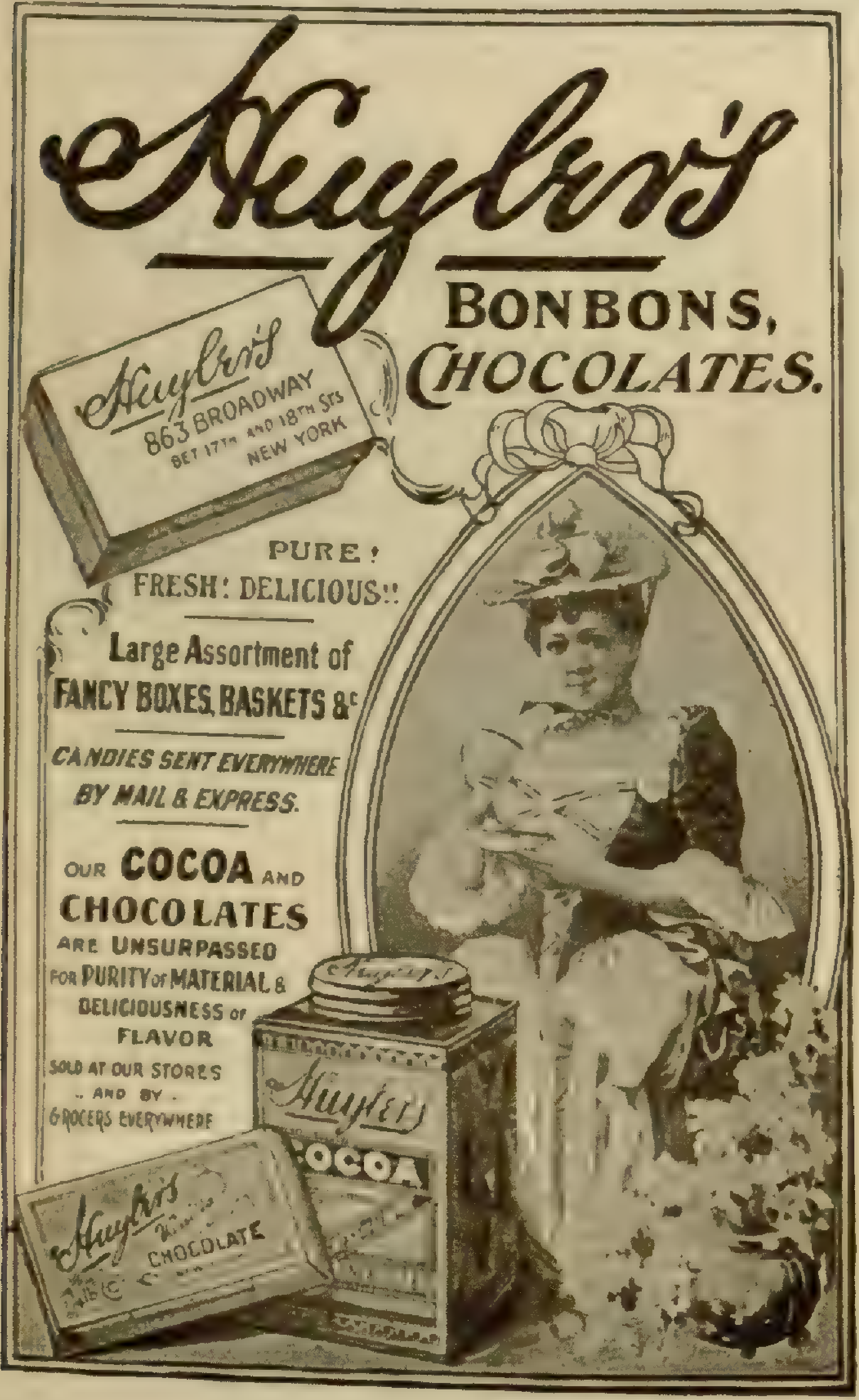

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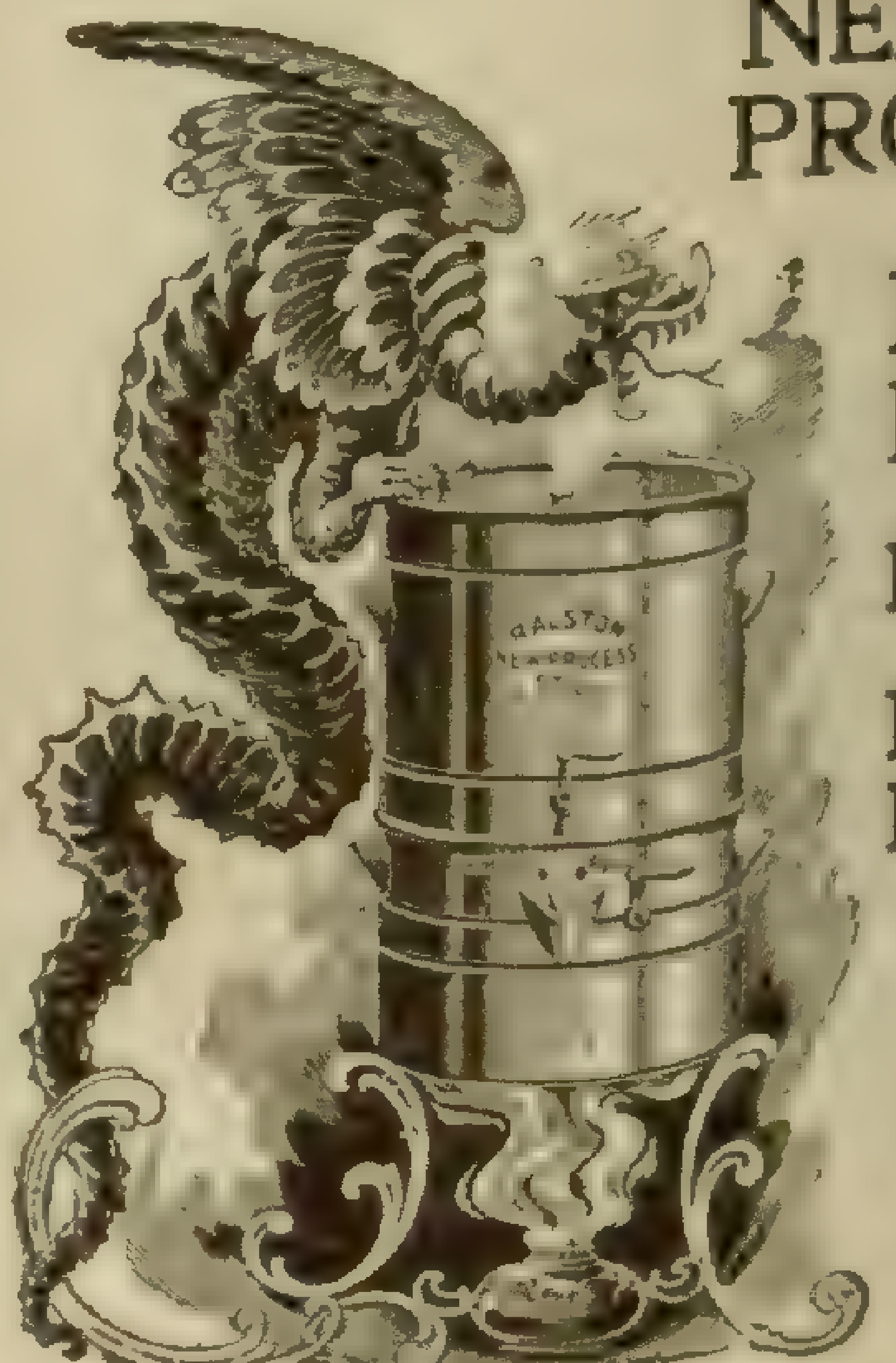

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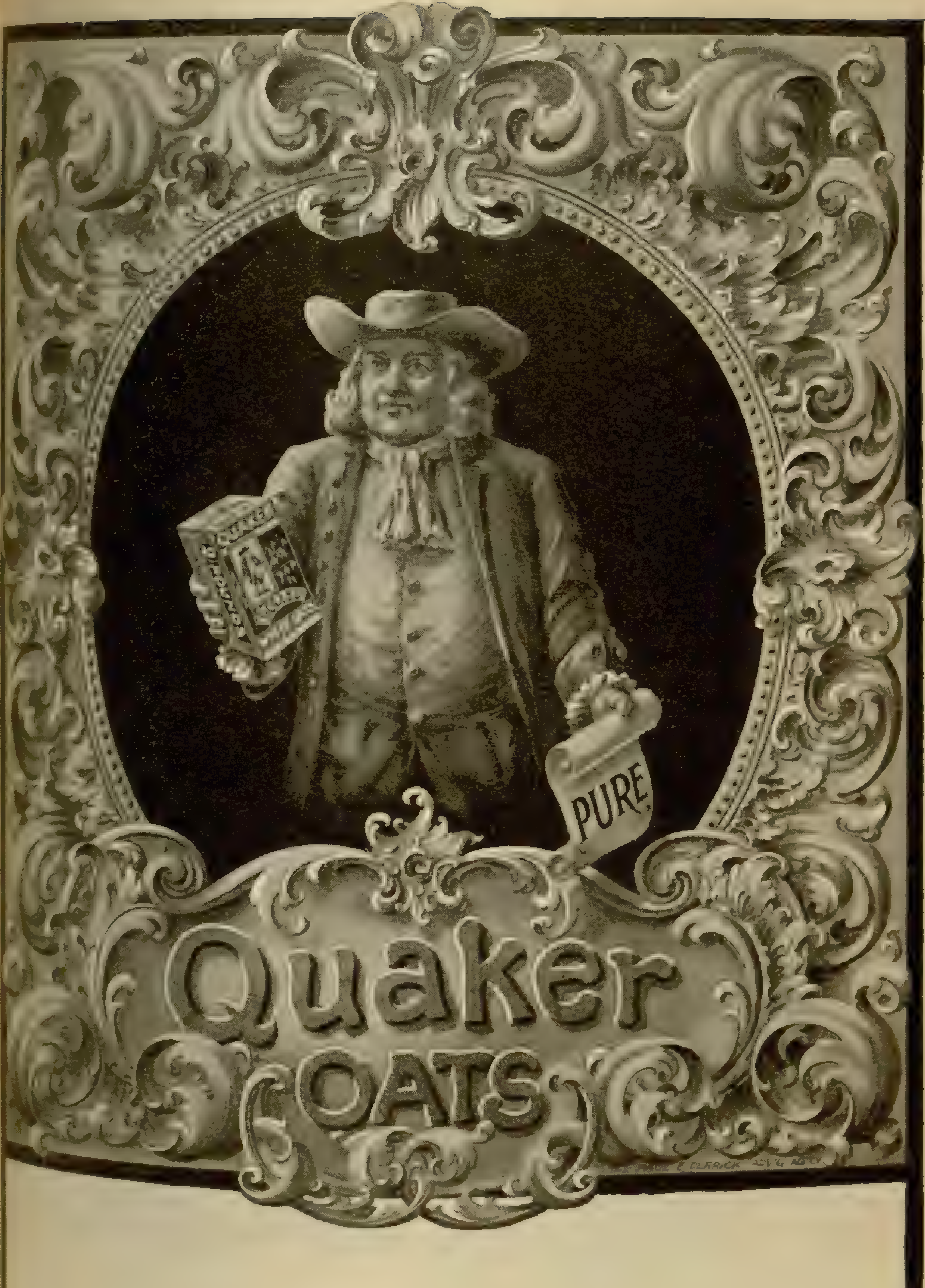
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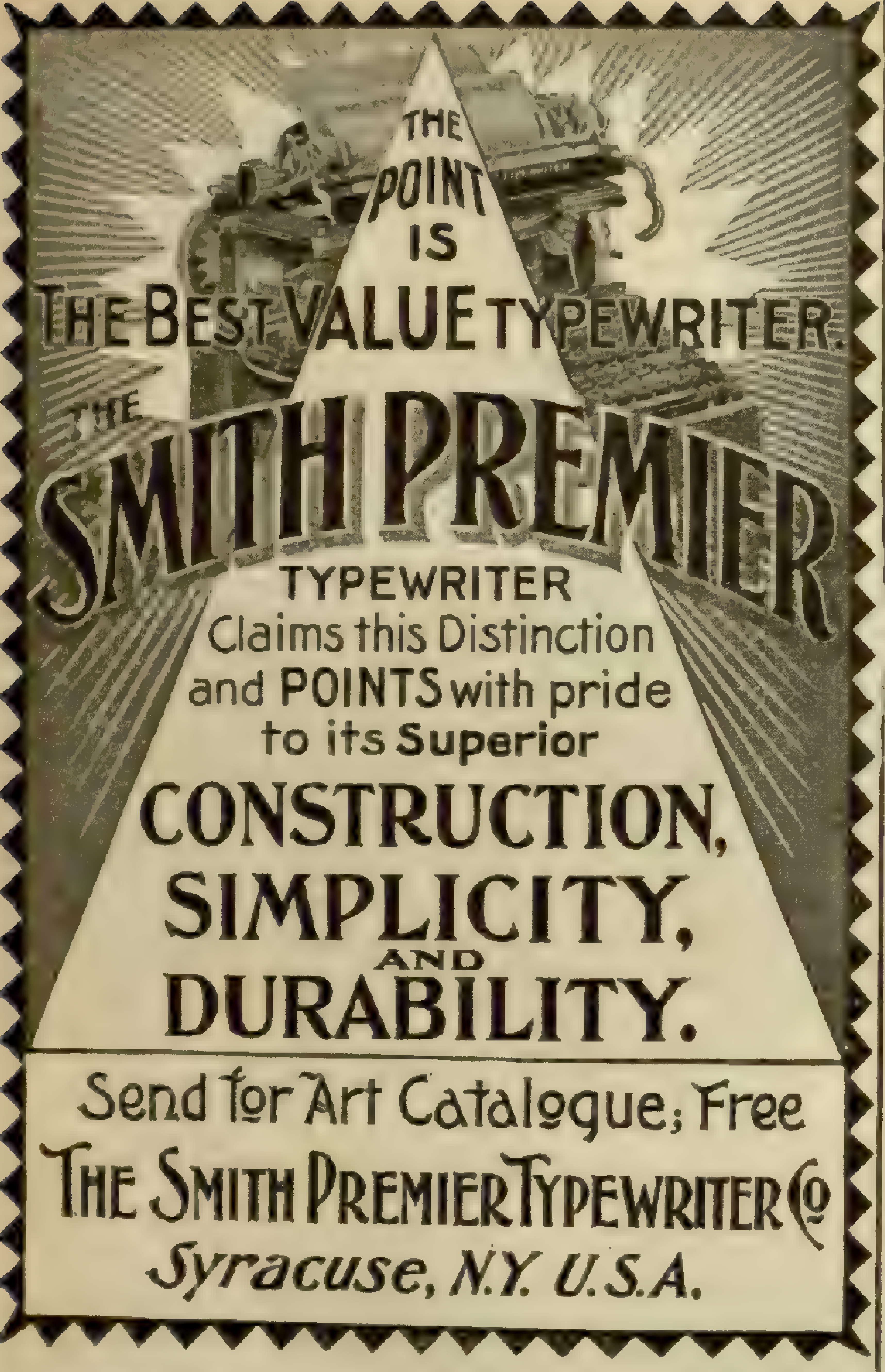

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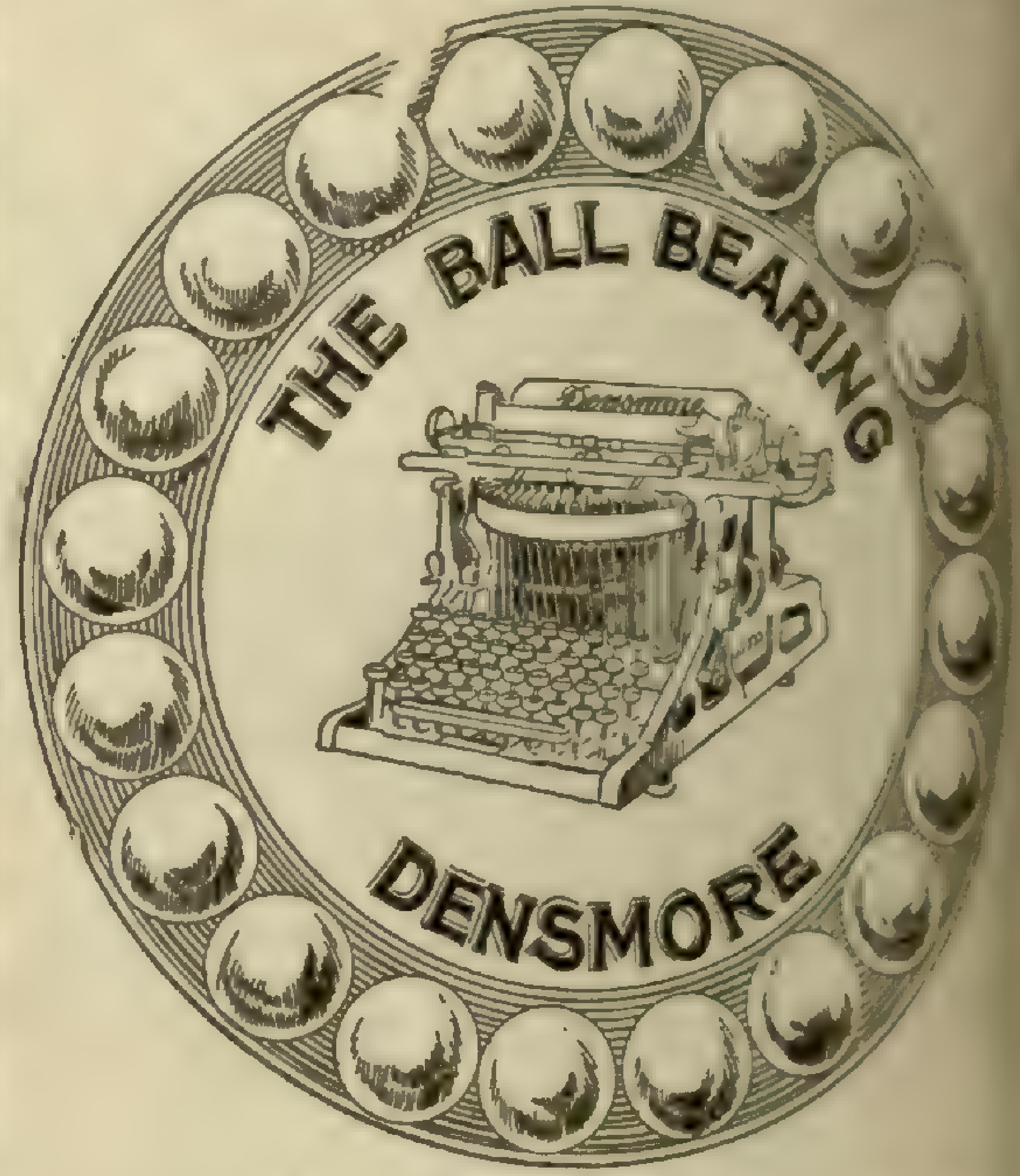

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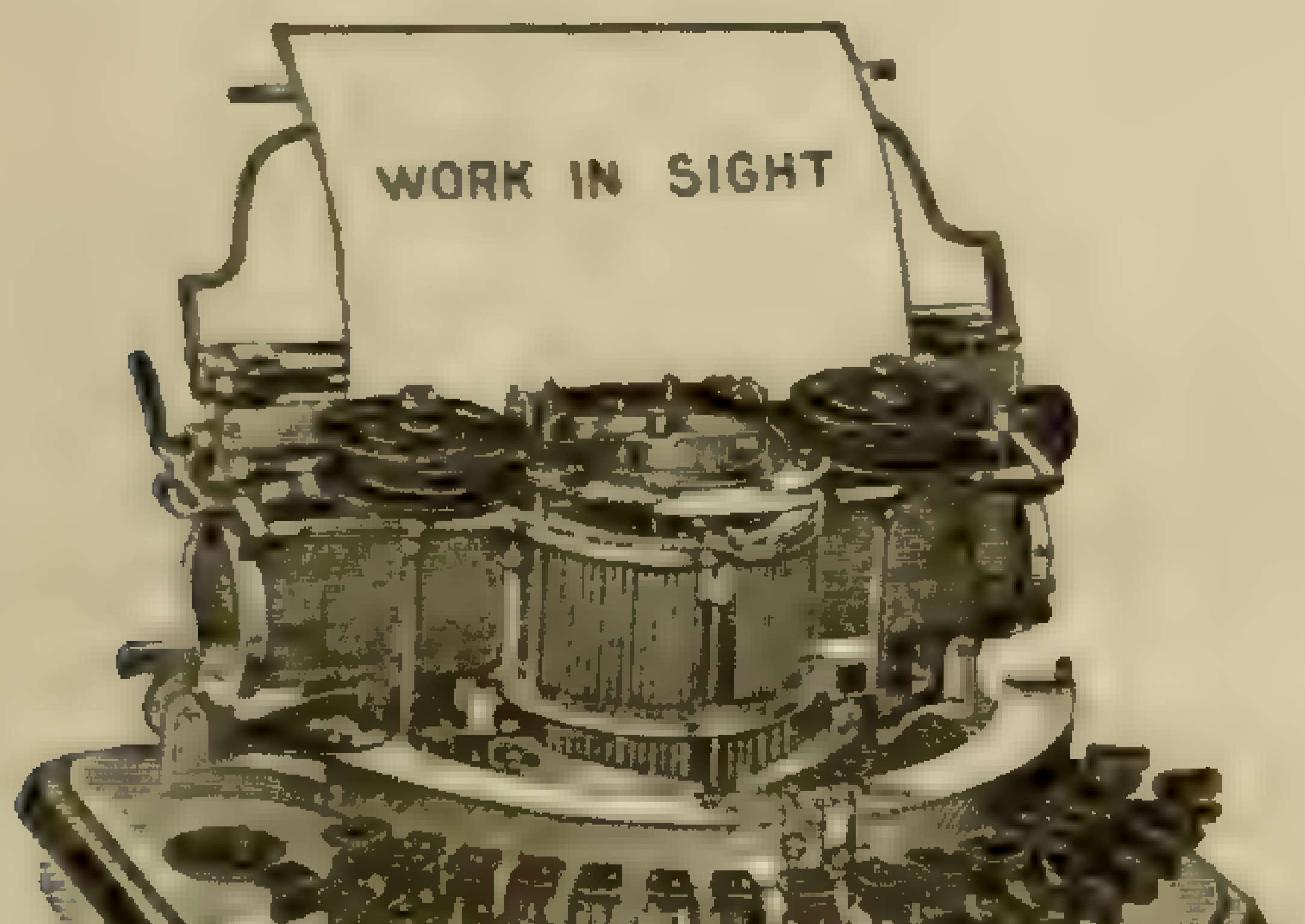

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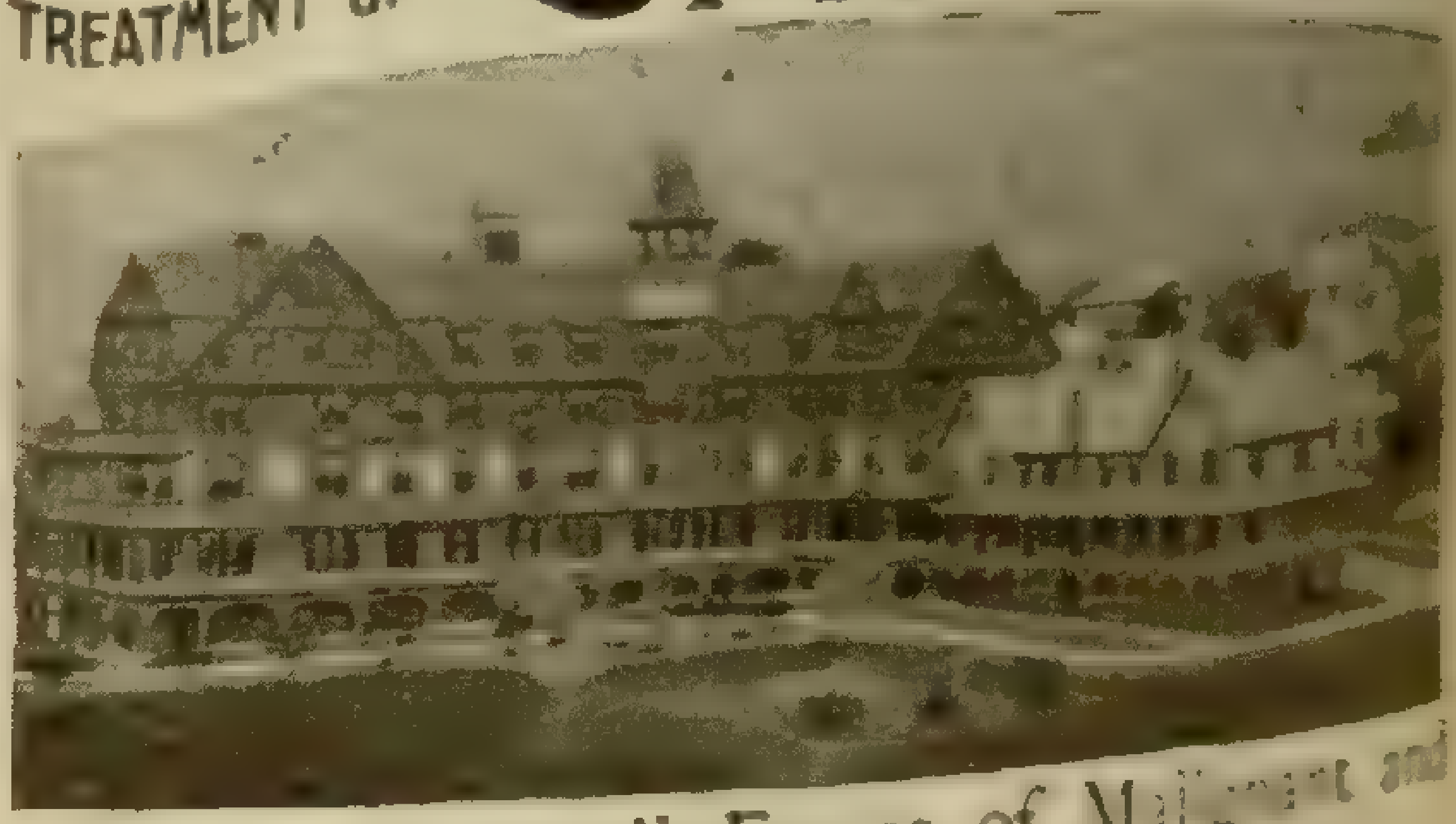

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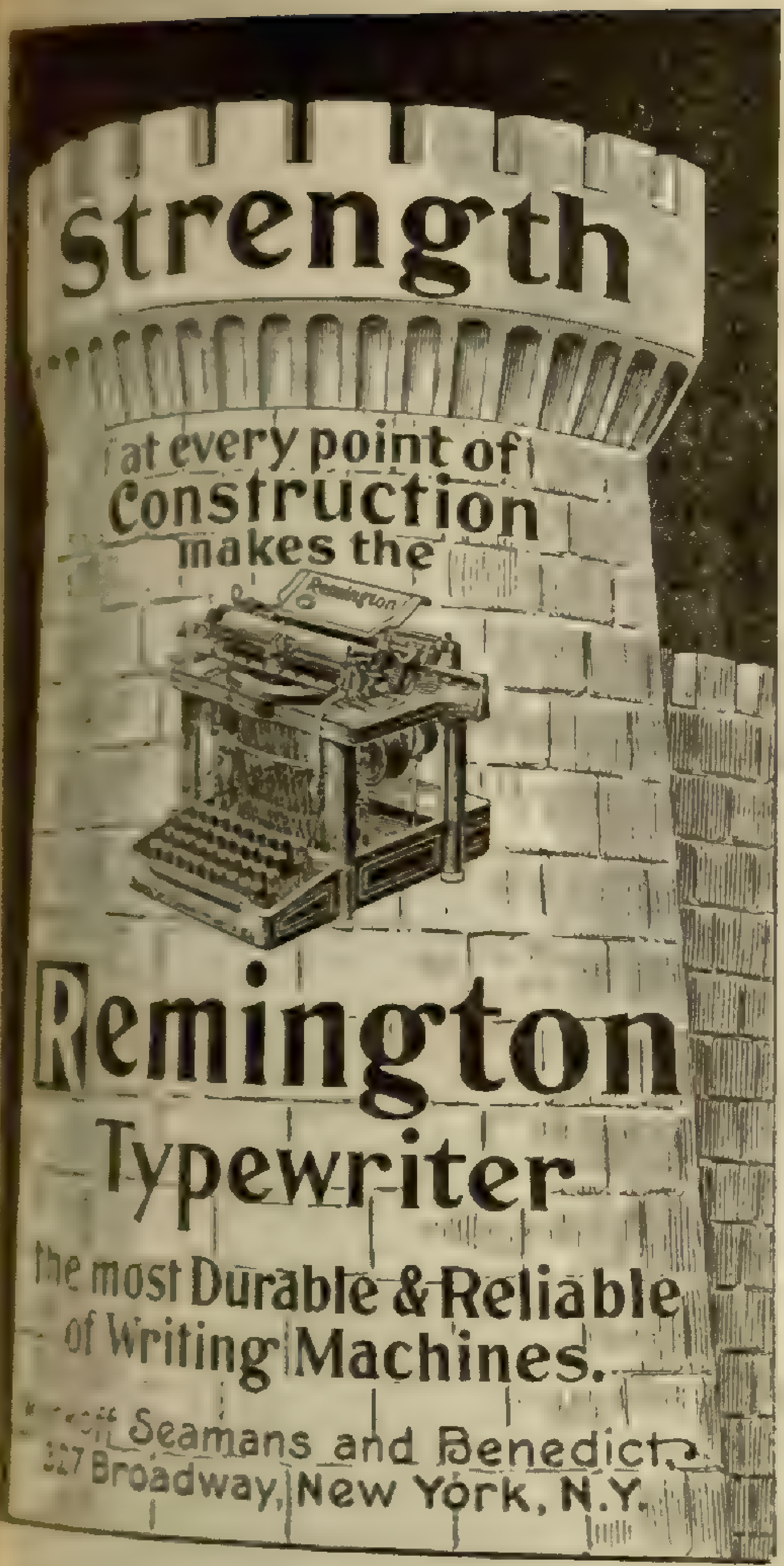

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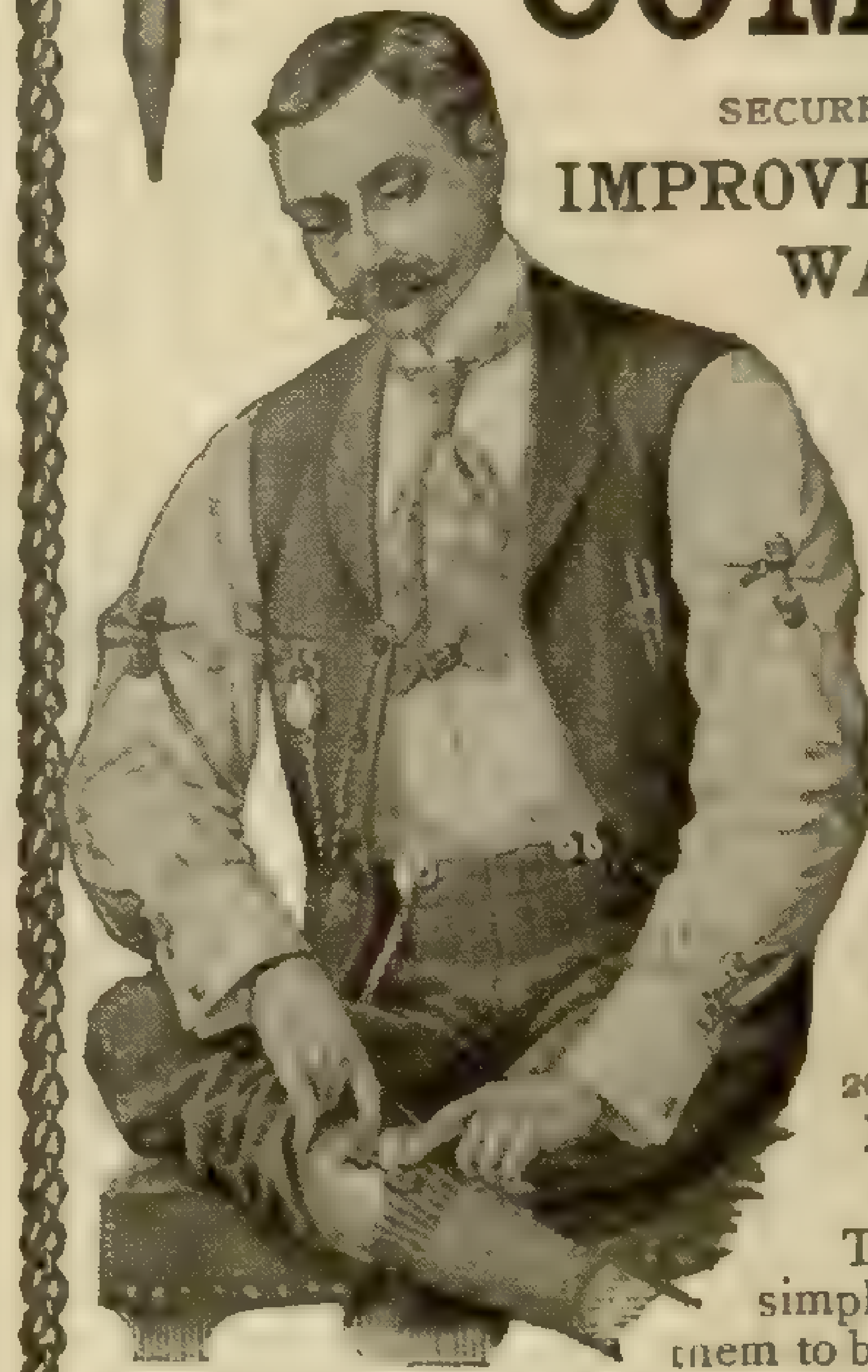

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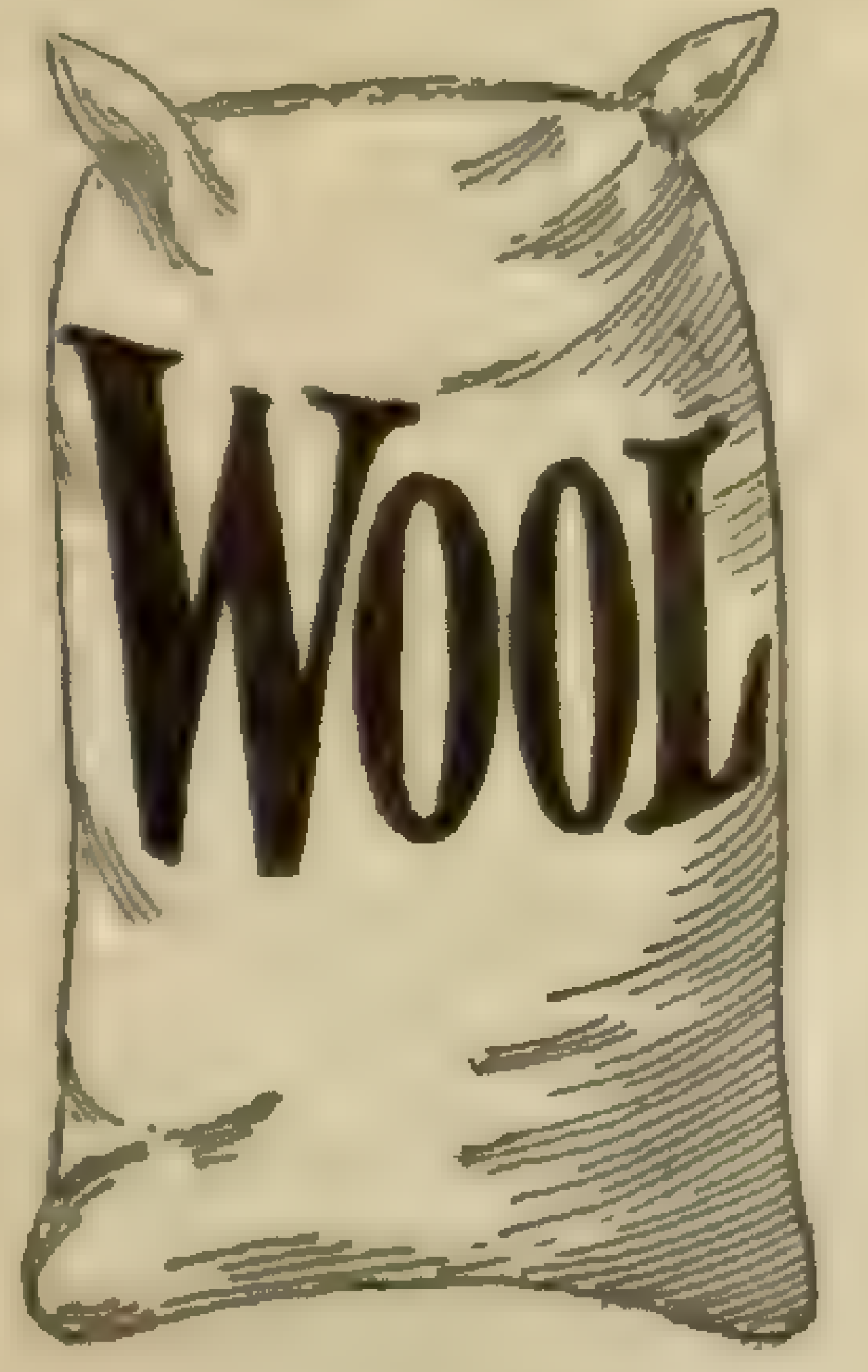

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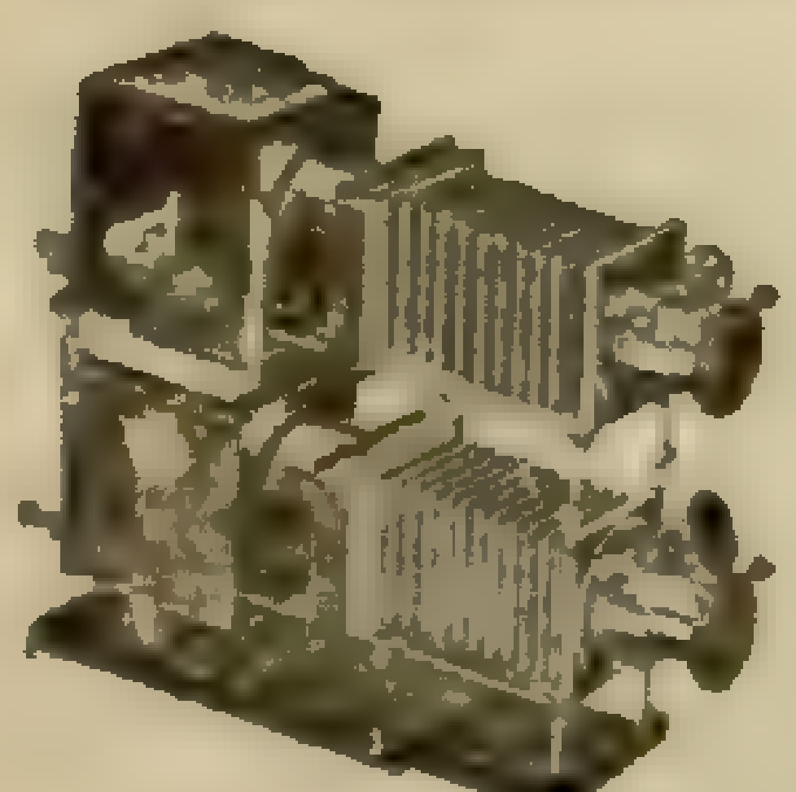

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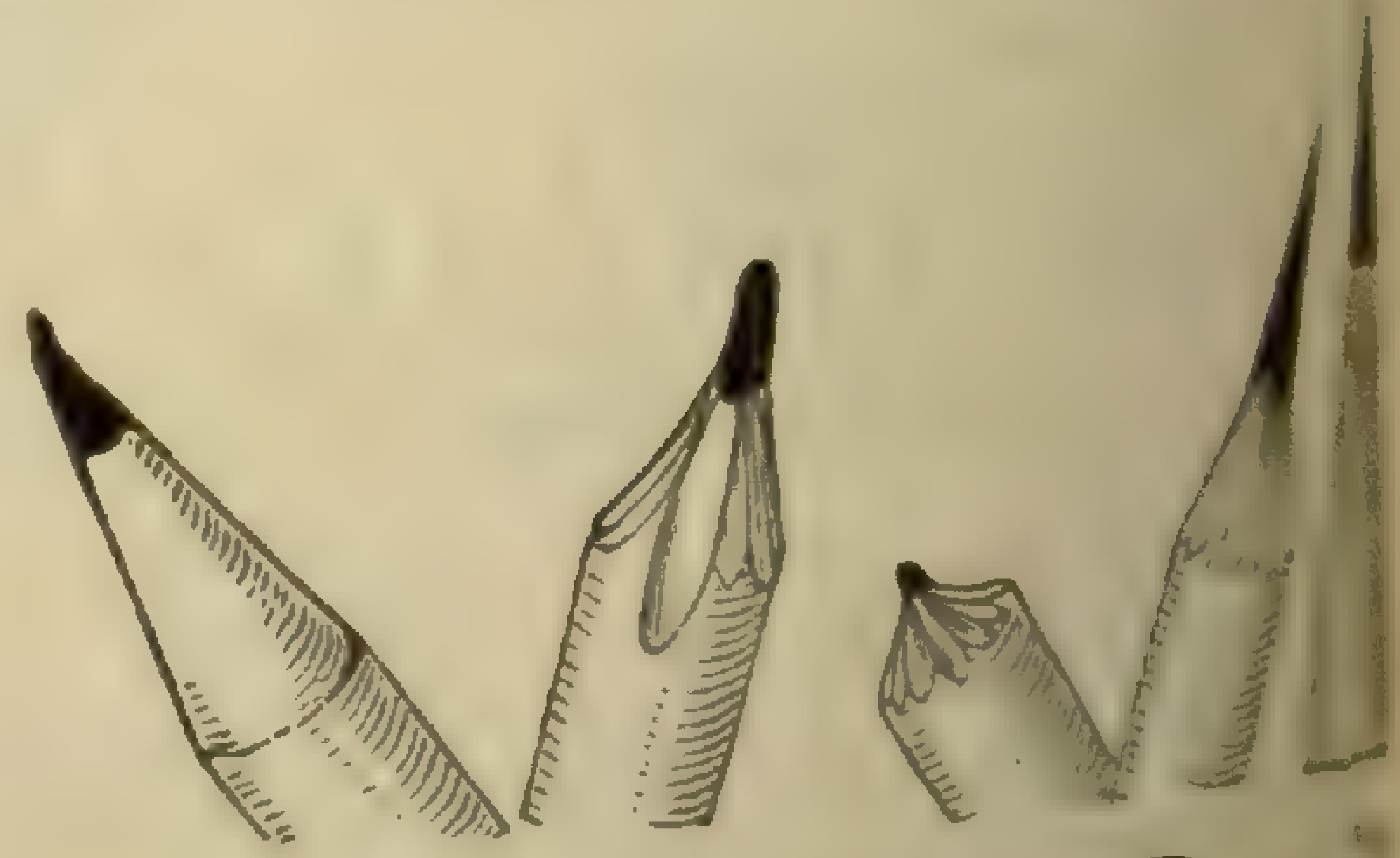

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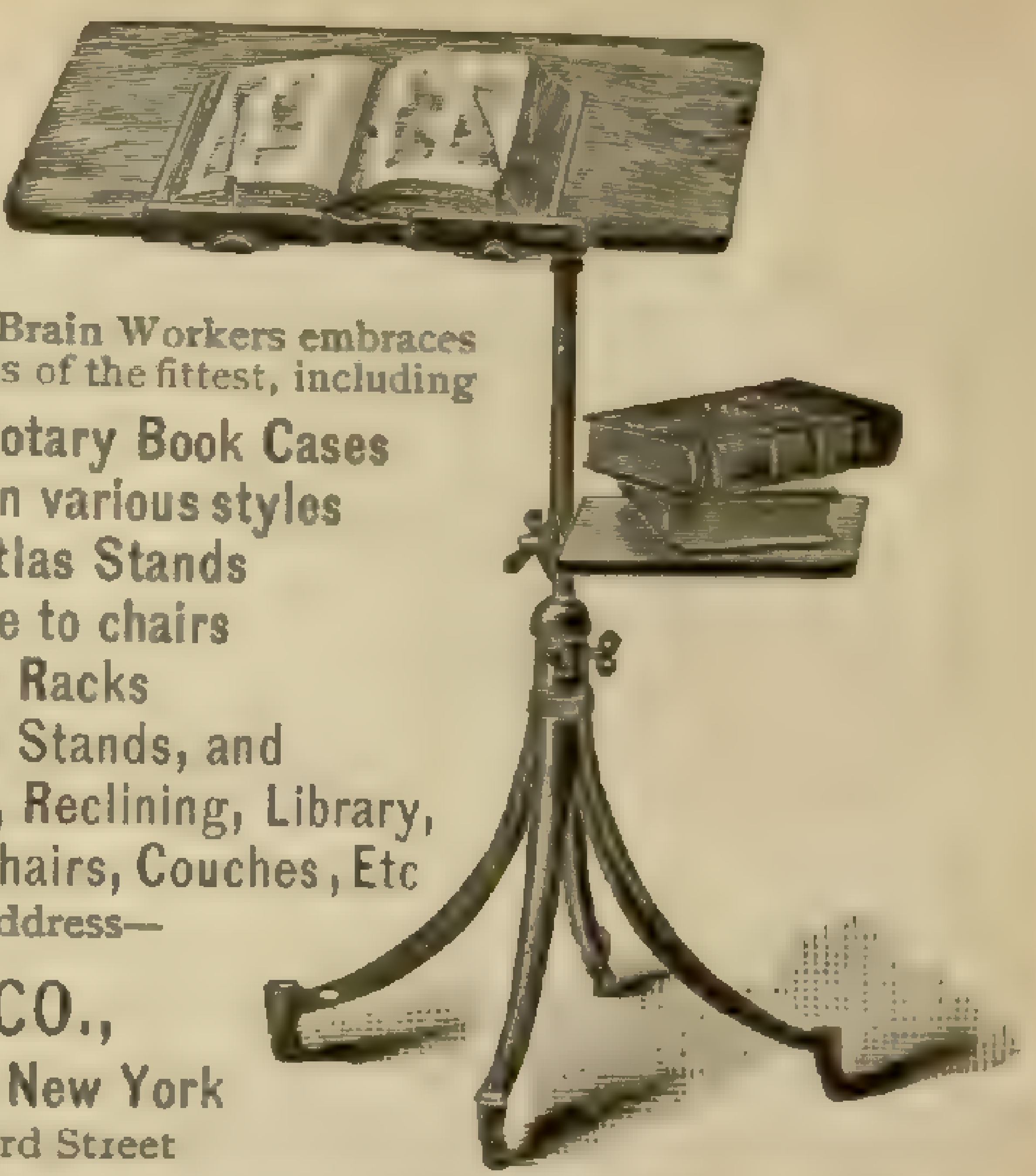

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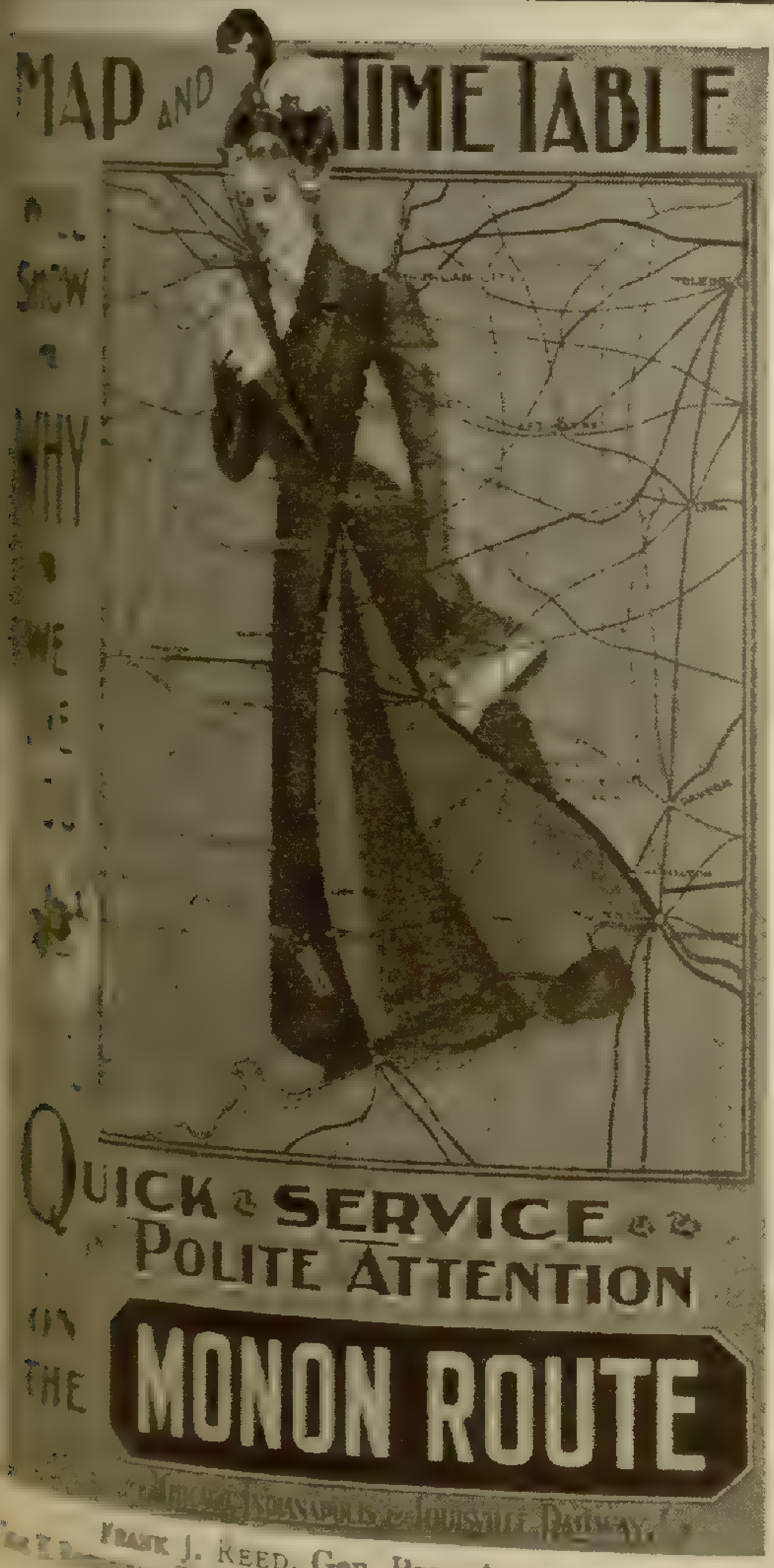

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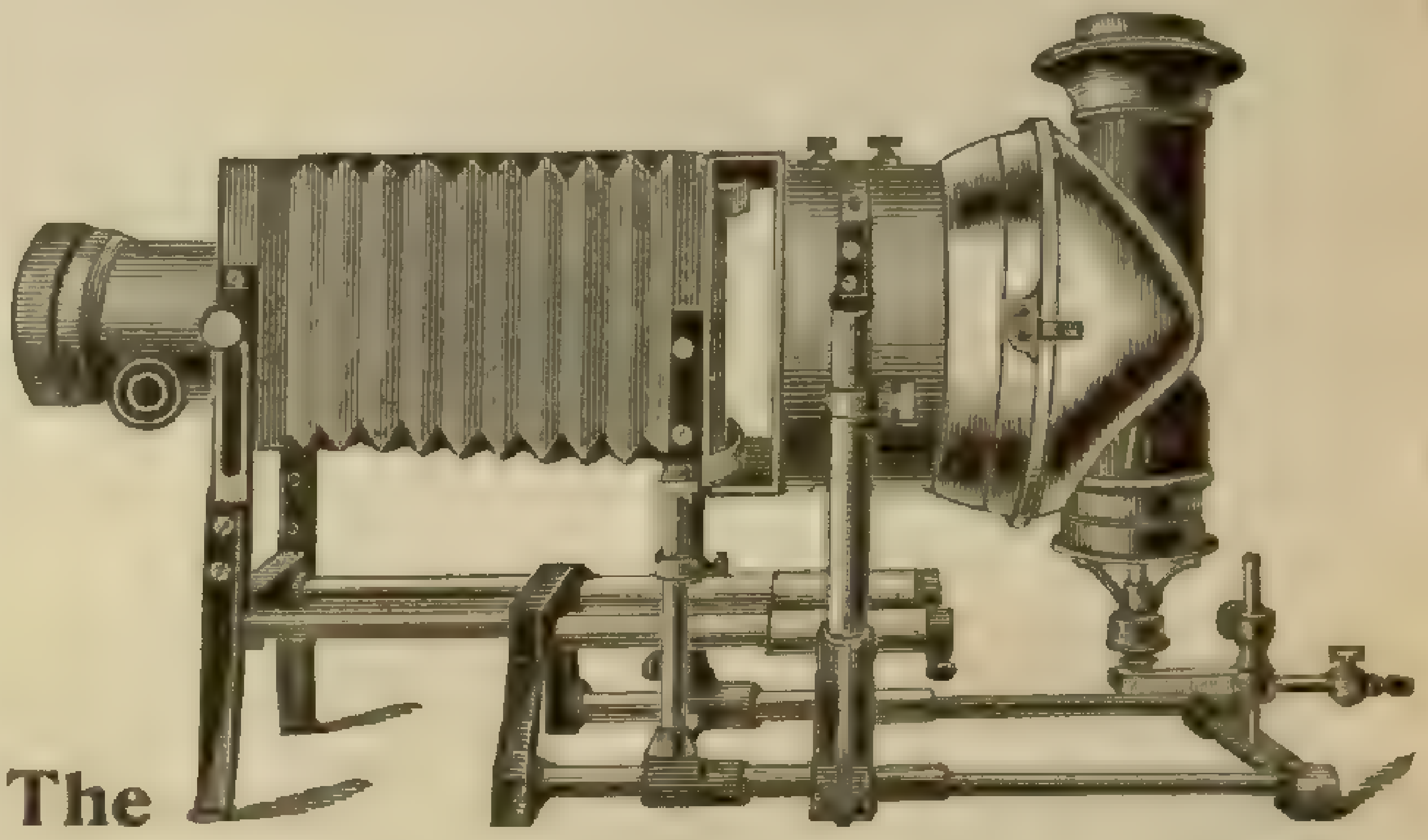

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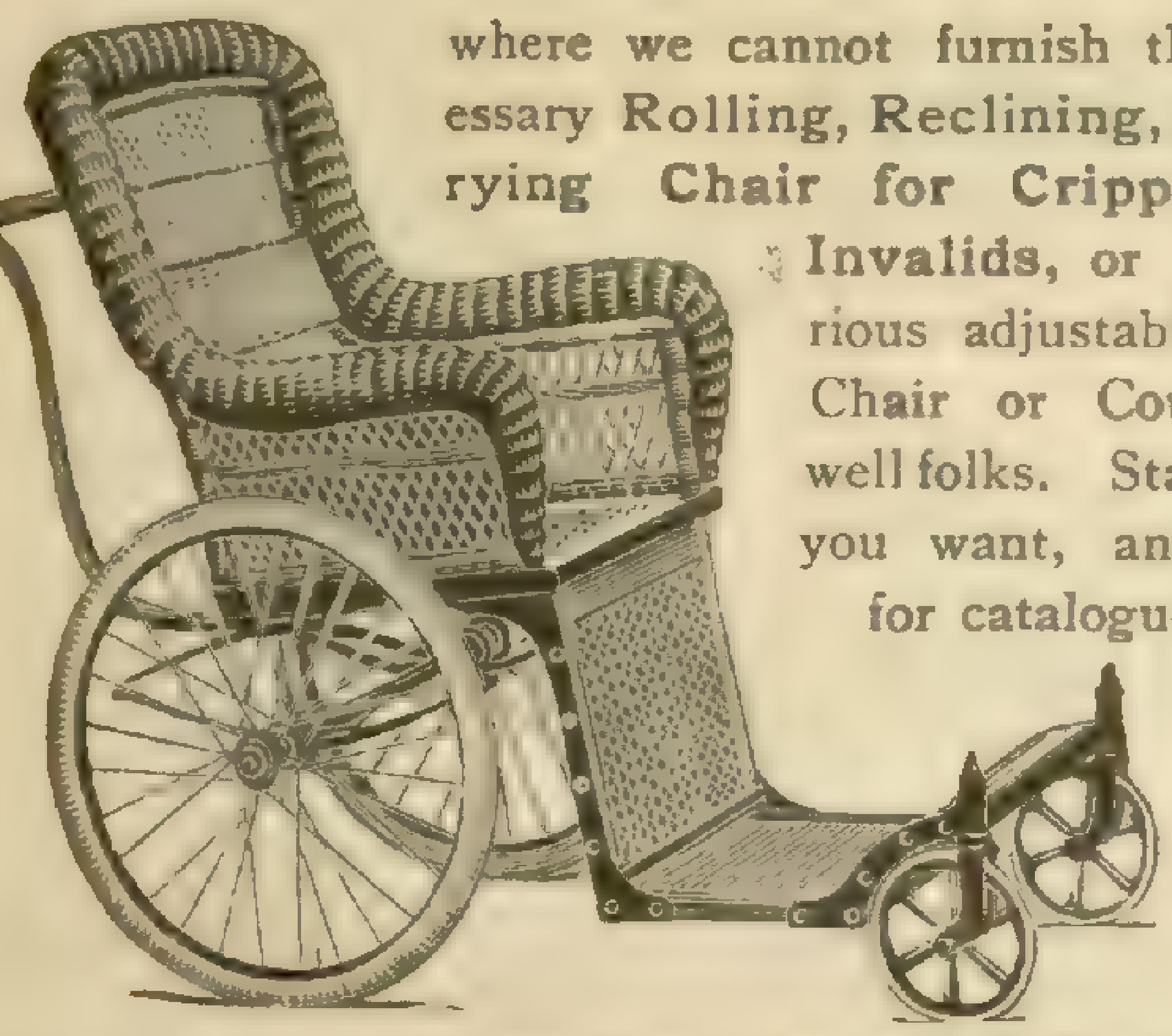

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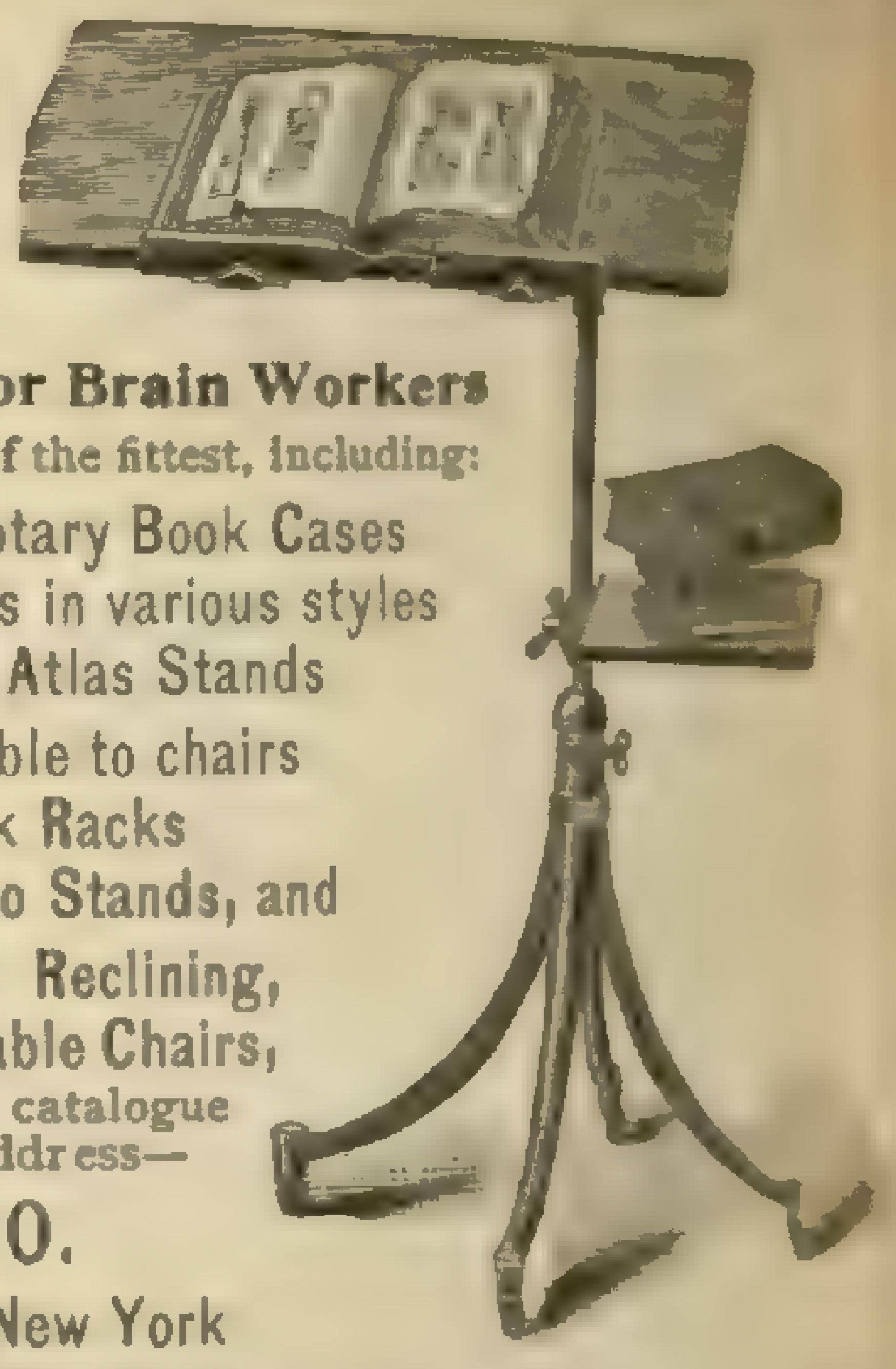

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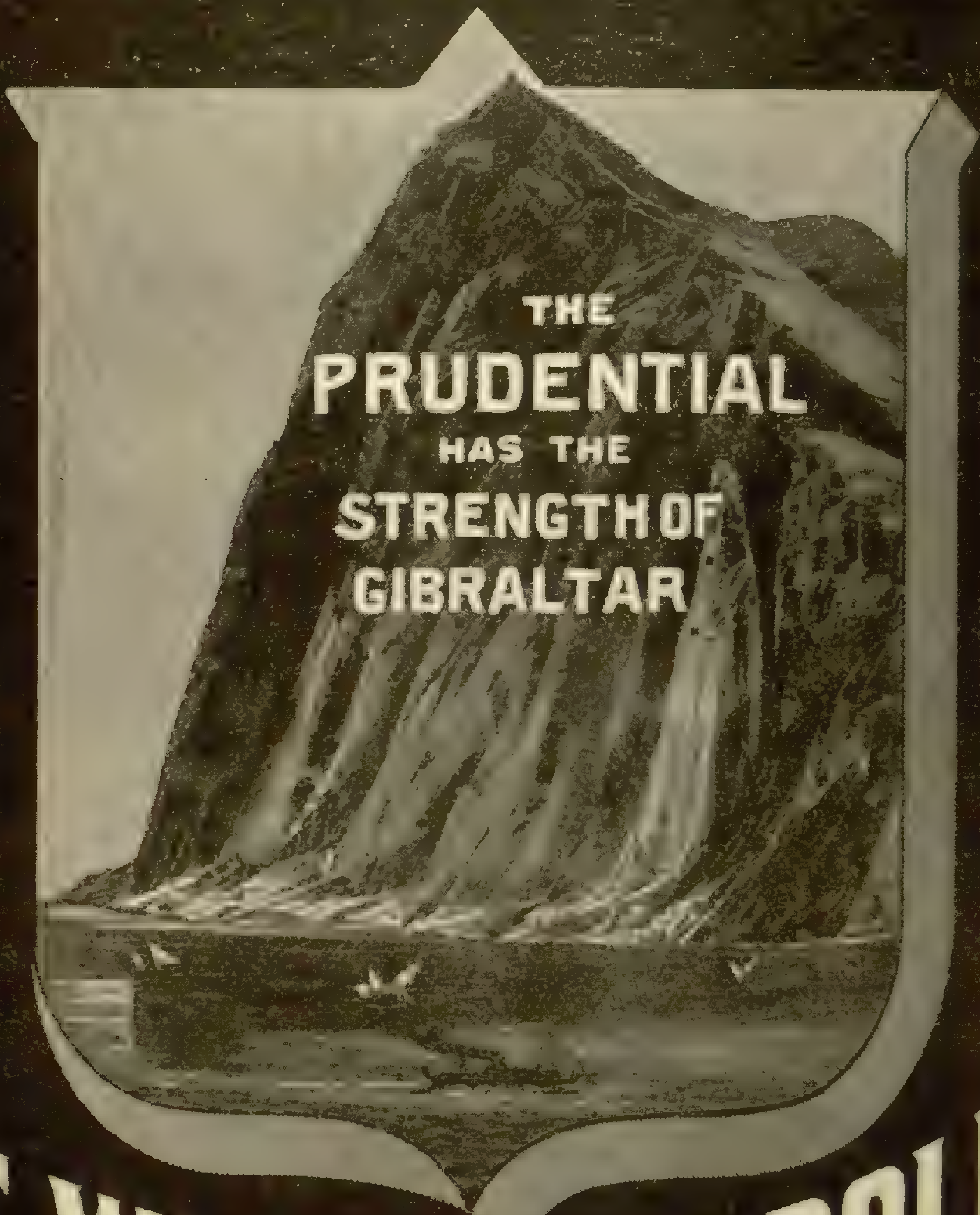

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OCTOBER 1899

THE COMPOUND OOSPHERE OF ALBUGO BLITI.

CONTRIBUTION FROM THE HULL BOTANICAL LABORATORY XVI.

F. L. STEVENS.

[*Concluded from p. 176.*]

RIPENING OF THE OOSPORE.

THE process by which the oospore attains its mature condition is easily divided into three stages, which are marked by changes that take place in the oospore wall, as follows: (1) a period during which the exospore is partially constructed, and which terminates with the beginning of (2) when the primary endospore is laid down and the exospore nearly completed, and (3) a period during which the secondary endospore is formed and the oospore becomes fully ripe and ready for its winter rest.

The nuclei in the oosphere withdraw from the immediate vicinity of the primitive wall soon after fusion of the sex nuclei, even before this act is completed, and the strands of cytoplasm toward the center thicken, leaving those of the periplasm but no more than slender supporting threads. This condition is difficult to describe but is well represented in *fig. 89*. The nuclei have previously enlarged and the cytoplasm is more granular than at any earlier stage. The primitive wall becomes about 0.75μ thick.

At this time a peculiar substance appears in the interstices of the ooplasm and fills the large vacuoles. It is not seen in younger stages, but is constant at this period and takes a rusty color when Flemming's triple stain is used. When the substance first appears the tint is barely perceptible, but later it assumes a brick-red color. Slightly stained sections show a film in the vacuoles often so curled up that an edge view is obtained, thus showing that the vacuoles actually contain a stainable substance. This substance is present in stages such as those represented in *figs. 91, 92, 93, 94*.

As the primitive wall reaches maturity it becomes covered by a layer of semi-transparent substance which is penetrated by straight pores. The condition is illustrated in *fig. 92*, where a portion of this layer lies in such a position that both a surface and an edge view appear at one time. As the pores are close together and straight the structure reminds one of the hymenial surface of the Polyporei. Since the main substance of the layer is nearly invisible, the pores appear dark and look like papillae or cilia growing out of the primitive wall when seen in edge-view (*fig. 92*). A condition was noted by Wager in *A. candidus*, and referred to as the "columnar condition," which from the descriptions and drawings seems similar to this. There appear somewhat later in this semi-transparent layer peculiar saucer-shaped masses of a dark substance of unknown nature (*fig. 91*). They are the first indication of the ridges so characteristic of the mature epispore. There is but little periplasm at this period, although the nuclei of the periplasm are apparently still undiminished in number. However, they stain very lightly and show only a membrane and nucleolus. They frequently mass together in bunches, but there is no evidence that they disorganize to furnish material for the exospore as is described for *A. candidus*.

To complete the endospore the saucer-shaped regions, previously mentioned (*fig. 91*), extend laterally and run together, thus forming the ridges. This gives the epispore the structure shown in *fig. 93*. Nuclei persist much longer outside of the exospore, but they seem to be functionless, and their history was not followed.

other. The mature endospore consists of two layers that may be called the primary and secondary endospores according to their development. The primary endospore appears as an even layer lining the primitive wall, at first agreeing precisely in stain reaction with the gelatinous material of the vacuoles, which is present (*fig. 93*). The primary endospore, however, soon loses this stain and gives the reaction of cellulose with chloride of zinc. A variation in the normal sequence was found in one oospore in that the epispore was completed before any trace of endospore was to be seen.

The conspicuous feature of the third period is the laying down of the secondary endospore inside of the first. It is of cellulose, and is about equal to the primary endospore in thickness. The two layers of the fully developed endospore are clearly evident when stained, also when they cleave apart in a perfectly regular manner as they frequently do (*fig. 97*). There seems to be a pause in the laying down of cellulose when the primary endospore is completed, as is shown by the fact that perceptible changes occur in the development of the epispore after the first and before the second endospore is formed. *Fig. 94* shows the earliest trace of the secondary endospore that was seen. When the second layer of cellulose is completed the gelatinous material has completely disappeared from the vacuoles. The fact that this substance appears just before the laying down of the primary endospore, the agreement in their staining reactions while the endospore is young, and the disappearance of all of the gelatinous material simultaneously with the completion of the secondary endospore, are significant facts. They indicate that this substance is intimately connected with the formation of the cellulose walls of the spore. It appears to be transferred directly from the vacuoles to the exterior of the protoplasm, there to change to cellulose.

Finally, various food substances accumulate in the center of the oospore, taking the form of one very large irregularly shaped globule (*fig. 97*). Around this mass lies a zone of cytoplasm containing the fusion-nuclei in resting condition. Their number is about one hundred, so it is not probable that there has been any multiplication since fertilization. Mitotic figures have never been seen in the oospore after that act, and it is probable that the oospore persists in this condition until the following spring.

OILS AND OTHER FOODS.

A thorough discussion of this subject would involve a much more elaborate microchemical study than was undertaken. It seems desirable, however, to record a few observations since these extra-protoplasmic substances are very conspicuous at certain periods of development and their presence often introduces serious difficulties of technique. No writer has given the subject the attention that it deserves, and detailed study would doubtless yield valuable results.

There are three substances which, when present, always appear as nearly spherical globules with the general appearance of oil, and they have undoubtedly been described as such by many previous observers. Further study may show that they are not true oils, and this seems quite probable, as they do not answer to all of the microchemical tests; but in the lack of a precise knowledge of their chemical nature they may be considered here under that name. All of them are found in the serial sections cut from paraffin, that is, in material which has passed in bulk through the weak alcohols and rested in 75 per cent. alcohol for weeks or months; which has passed to the paraffin through chloroform and been cleared of paraffin by immersion for a few moments in xylol; and finally which has undergone the baths accessory to the stain employed.

The first oil is very abundant, existing in far greater quantity than either of the others, and during more stages in the development of the fungus. It is found in the young oogonium, the oosphere, and the periplasm. Diagrams showing its distribution in the important periods of development are given in the plates. *Fig. 46* presents it for an oogonium of about the age shown in *fig. 45*. *Fig. 63* illustrates its distribution at zonation, and it should be noticed that the drops are small where the meshes of the ooplasm are fine, but large in the periplasm where the meshes are more coarse. *Fig. 72* shows the condition of an oogonium at a stage similar to *fig. 70*, a period somewhat later than zonation. The distribution is much the same because the protoplasm has changed but little. *Fig. 81*, a stage after fertilization, indicates that as the meshes of the ooplasm become coarser the globules become larger; apparently some of the globules fuse to form the larger drops. A later stage similar to *fig. 93* shows that the oil of the periplasm is nearly exhausted during the building up of the epispore.

This most common oil is found in unstained sections as blackish or brown drops when the material has been killed by Flemming's fixing fluid. It is usually absent from material fixed in chrom-acetic acid, or if present is in very small quantity. If the material is allowed to remain several (six) days in the chrom-acetic fixing agent the oil appears in considerable quantity, very much as it does in Flemming's material, but of lighter straw color instead of brown. When material fails to show the oil it seems to be really absent, because several days' immersion in 1 per cent. osmic acid solution fails to show its presence. Therefore, it seems that the osmic acid of the Flemming's fixing fluid, or the long immersion in chrom-acetic acid, so acts on the oil as to render it less easily soluble in the fluids that it meets between the killing and the time when the sections are stained. This oil when present takes a characteristic and beautiful red color from the safranin in the Flemming's triple stain combination. If it be very dark from the action of the osmic acid, it is frequently necessary to employ hydrogen peroxid to remove the blackness, in order that the best results may be obtained from the stains. It is partially on account of this oil that material killed by Flemming's fluid is not so favorable for cytological study as that fixed by the chrom-acetic acid.

The second oil-like substance is present only in very small quantity, possibly eight or ten small globules in an oogonium. It is evident in both Flemming and chrom-acetic material, is stained black by Heidenhain's hæmatoxylin, but may be distinguished from the first oil by the fact that it does not take the safranin when applied in the usual way. It is figured in the plates as black dots (*figs. 51, 56, 59, 61, 64, 68, 69*); in the antheridium in *fig. 85;* in the periplasm in *fig. 80.* No difference in stain reaction was observed between this oil-like substance and the central globule of the cœnocentrum, and they may be of similar nature.

The third oil is only found in the maturing oospore, first appearing while the secondary endospore wall is developing as globules of a clear honey-yellow color in the meshes of the protoplasm (*fig. 95*). It soon accumulates at the wall, and *fig. 94* shows large drops in close contact with the forming secondary endospore, which has broken away from the primary endospore, probably owing to the impact of the knife in cutting. The drops finally become larger and more numerous, as is shown in *fig. 9*[illegible] From a study of subsequent stages it seems probable that these oil drops later break away from the wall irregularly, and unite to form the several large drops that are often found in older oospores. This results finally in the condition shown in *fig.* [illegible] where the entire central region is occupied by a curious irregular globular structure which stains much as the oils have stained in previous stages, but which is certainly not of fluid consistency. It probably represents reserve food material. If this structure represents a genuine oil drop in the living spore, we may have to do here with the shrunken and collapsed vesicle or membrane that encased it in life, and whose contents have been lost during the processes of technique. Its appearance would accord well with this view. From its size and the time of development in the oospore it is surely the structure described as oil by previous investigators, and is characteristic of the winter oospore.

GENERAL CONSIDERATIONS.

It seems well before closing to take a general view of the facts that have been presented, and of their relation to previous knowledge; also to point out more clearly than was possible in earlier pages the broader significance of the phenomena described.

A mitotic division of the nuclei of fungi seems to have been first noticed by Sadeback ('83) in Exoascus, and has since been observed in various forms and carefully studied and described in a few papers.

The occurrence of a mitotic division in the oogonium of Albugo was discovered by Wager ('96) in *A. candidus*, and confirmed by Berlese ('98) in *A. Portulacæ*. Wager notes that the nuclei enlarge and divide, leaving one daughter nucleus imbedded in the central body while the other nuclei pass to the periplasm. The one division increases the nuclei from about 115 to about double that number. Berlese says the nuclei divide several times in *A. Portulacæ*, increasing the number from 30 or 40 to about 200. The account given in the present paper describes two approximately simultaneous divisions affecting all of the nuclei in both antheridium and oogonium, and these mitoses result in the formation of the sexual elements, numerous male and female nuclei. The second division is strikingly different from the first in the appearance of the nuclear elements, particularly the chromatin. This condition suggested the possibility of a reduction of chromatin, but careful study revealed no convincing evidence. The mitoses are characterized by the intranuclear formation of the spindle, the intranuclear centrosomes, the permanence of the nucleolus, and the entire absence of extranuclear radiation.

Wager described the disappearance of the nucleolus in early prophase, followed by the formation of chromosomes and then by the spindle development. He inclines toward the view that the spindle is derived from the linin. The membrane persists up to metaphase, and Wager did not follow the division further. Berlese, from observations similar to those of Wager, namely, the disappearance of the nucleolus in prophase, argues that the spindle fibers are derived from it. The behavior of the nucleolus in *A. Bliti* is different from that described by the writers mentioned, in that the structure remains apparent inside of the nucleus through all stages of mitosis. However, since Wager studied this question only incidentally, and as Berlese gives no figures and his account is very brief, a detailed comparison of the species is impossible. Berlese reports from twelve to sixteen chromosomes, and seems to have been able to count them during the fusion of the sexual nuclei. Neither Berlese nor Wager give details of the mitosis later than prophase.

Wager reports that as near as he can estimate there are from twelve to sixteen chromosomes shown in the mitotic figures. In *A. Bliti* six are found with certainty in some anaphase nuclei, and twelve appear in some metaphase nuclei with equal certainty. It may be that when twelve are counted the chromosomes have already divided, and that they really belong to two rather than to one nucleus. However, this is not certain, and there is some evidence that makes it appear that there is a reduction in the number of chromosomes during the first mitosis, but this cannot be considered as proved.

Centrosomes were not observed in either *A. candidus* or *A. Portulacæ*, or in any other of the Phycomycetes, so far as the writer is aware; but they have been described in earlier papers for Ascomycetes (Gjurasin '93, Harper '95), and for Basidomycetes (Wager '92, Juel '98).

With such fundamental differences as have been indicated, it is useless to attempt to establish a type of mitosis for Albugo, or to attempt to determine the relationship of the group through cytology. The type here described for *A. Bliti*, while presenting a few deviations from the mode of mitosis in vogue among higher plants, is in no way a departure from the forms well known among the lower types of plants and animals.

It is impossible to generalize on the facts of morphology presented in this paper, because simple processes of fertilization have been described in all forms where homology would be sought. Until the behavior of the nuclei of other species of Albugo is known, it is impossible to say which is the anomalous form of the genus, *A. candidus* or *A. Bliti*. From a preliminary study of *A. candidus* it can be affirmed that *A. Bliti* is in most ways far more favorable for study; its nuclei are larger, its periplasm more abundant, its developmental stages more strongly marked, and its antheridial tube larger. *Albugo candidus*, however, has a remarkable cœnocentrum, which will be much easier to study than that of *A. Bliti*, owing to its much greater size and more pronounced stain reaction. A problem of great importance lies in a comparative study of the cœnocentra of the genus.

The characteristic massing of the cytoplasm to form a rudimentary oosphere in *A. Bliti*, differing thus from the vacuolate oosphere of *A. candidus*, is not a wider divergence than might be expected in different species; nor is the variation in the cœnocentrum more than what might be regarded as a specific difference. If such variations are found to be more marked in other species the way may be clear to trace the relationship between plants with one oosphere and those with several oospheres in each oogonium; between forms which differentiate their periplasm after the manner of Vaucheria, and others that follow the habit of the Saprolegniaceæ in a parietal rather than a central massing. It must be left to future research to make clear the relationship that must exist between the multiple fertilization illustrated by *Albugo Bliti* and simple acts of fusion between sexual elements.

It may be that cytological investigation will show remarkable variations in many respects in this genus, and establish a chain of derivative forms. The Saprolegniaceæ are said to range from parthenogenesis to complete sexual fertilization. Should Albugo prove to be similarly rich in habits the present knowledge of relationships will be much increased.

METHODS, MATERIALS, AND STAINING REACTIONS.

The material upon which this investigation was based consisted of leaves, stems, and flower clusters of *Amaranthus retroflexus* L. and *A. hybridus* L. bearing the fungus. It was collected at Syracuse, N. Y., Columbus, Ohio, and Chicago, Ill. In all cases the species seemed to be unquestionably *Albugo Bliti* Biv.

(*C. Amaranti* Schw. *C. amarantacearum* Zal.). The form described by Zalewski ('83) as a different species was not met. Oospores are very abundant on both leaves and stems, producing on the former characteristic blister-like patches that assume a blackish hue if the oospores ripen in sufficient quantity. In the stems their presence may be predicted from peculiar swellings, usually accompanied by a reddish coloration, the entire plant often being thus affected. In partially diseased plants the oospores are likely to be found in the inflorescence, which reacts much as does the stem, becoming swollen and red. The parts most favorable for study are the stems, but leaves and flowers often section more easily. In killing the material undesirable parts were cut away, and the portions apparently favorable were cut into small bits; leaves were scored, and stems and peduncles were cut in pieces about 2 to 4^{cm} long, deep incisions being made every 2^{mm} to give ready access to the killing agent. The killing with suitable solutions was apparently perfect, and was as good midway between incisions as where the solution immediately reached the tissue.

The killing agent giving the best results was chrom-acetic acid of the following formula: chromic acid 0.8 per cent., acetic acid 0.5 per cent. in water. The material was usually left in the solution from twelve to eighteen hours, then washed in five or six changes of water, allowing about two hours between changes. It was then successively transferred to 12, 25, 50, 75 per cent. alcohol, remaining about two hours in each grade, and was left in the last grade until it was practicable to imbed in paraffin. A variation in the above method, by which the material was left six days in chrom-acetic acid gave interesting results. It rendered the oil in the protoplasm much less soluble, but caused the loss of many of the details of the mitoses.

Flemming's chrom-osmic-acetic acid was employed in the same manner as the chrom-acetic acid, but was not so useful since the sections were very much darkened by the osmic acid thus necessitating elaborate methods of bleaching before a desirable stain could be obtained.

Other killing agents used were corrosive sublimate in saturated aqueous solution; hot corrosive-acetic-sublimate in alcohol; Carnoy's fluid; absolute alcohol; Hermann's fluid; and Merkel's fluid. Most of these gave far inferior results to that obtained by the chrom-acetic acid, and none surpassed it in effect.

In order to imbed in paraffin the material was transferred through 85, 95, and 100 per cent. alcohol to a mixture of absolute alcohol and chloroform, first of one third then of two thirds strength of chloroform. The specimens were left about two hours in each fluid, and were finally placed in pure chloroform. After the material had been left in chloroform for an hour a quantity of paraffin was added, and two hours later the material was warmed on the bath. After two hours of gentle heat it was removed to a warmer position, and later the most of the chloroform was poured off, melted paraffin substituted, and the whole kept in the bath at a temperature of about 55°. It was found advantageous during the whole process of imbedding to use very shallow dishes, as by this means most or all of the chloroform is driven off gradually by evaporation. Material was left in the bath in soft paraffin three or four hours, the paraffin being twice changed in the meantime to insure the removal of all chloroform. It was then cast in a cake, the final paraffin having a melting point of about 62°. Sections 3 to 5 μ in thickness were cut with a Jung sliding microtome and fastened to the slide with Mayer's albumen fixative.

Flemming's triple stain, used with chrom-acetic acid material, gave the best results. This stain demands the greatest attention in its use or failure is inevitable, as is well known by all who employ it. In general, the best results were attained by a bath of 30–60 min. in safranin, followed by a rinsing more or less prolonged in acid alcohol. The time here is entirely a matter of judgment, varying with the result desired; 30–90 sec. was the most usual time. After running down through the alcohols the slide was placed in saturated solution of gentian-violet for from 5–45 min. It was then rinsed in water and placed in orange

G from 5–25 sec.; a longer time may do no harm but probably 10–15 sec. is always sufficient. Wipe away excess of liquid and flood the slide twice with absolute alcohol, allowing the second lot of alcohol to remain on the slide until sufficient of the gentian-violet has been removed. The time required will depend on the material, the length of time it was in the gentian-violet, and the result desired. Drain rapidly with filter paper and flood with clove oil for one minute; drain, follow by cedar oil and cover in xylol balsam. If properly stained the host cell wall should be a light violet, the chromatin of the spirem and the chromosomes blue, nucleolus and centrosomes red, and cytoplasm slightly yellowish.

Hæmatoxylin stain was used, applied after the method of Heidenhain (iron-alum 2 hrs., hæmatoxylin 12–18 hrs., followed by the slow extraction of iron alum till the proper degree is reached). This treatment gave some beautiful results in contrast with the Flemming stain, and was particularly valuable in demonstrating the achromatic portion of the nuclear figure. Hartog's nigrosin-carmine stain, as used by Wager and Berlese, was tried repeatedly on corrosive sublimate material, but the results were far inferior to those afforded by Flemming's triple or Heidenhain's hæmatoxylin stains. However, it is possible to demonstrate, even with this combination, the presence of many nuclei in the oosphere and in the antheridial tube, and to recognize the principal features of the mitotic figures. Other stains employed were Delafeld's hæmatoxylin, Biondi-Ehrlich, and cyanin-erythrosin, but they were distinctly inferior in their results.

The following stain reactions were presented in the best preparations, and were attained by the Flemming's triple stain unless otherwise stated: chromatin blue or violet, black with Heidenhain's hæmatoxylin; nucleoli red, black with Heidenhain's hæmatoxylin; centrosomes as nucleoli; spindle fibers dark blue; cytoplasm yellowish; granules, mentioned on page [illegible] are only seen in preparations stained with Heidenhain's hæmatoxylin, and then black.

SUMMARY.

1. The oogonium when cut off from the parent hypha contains about 300 nuclei, which enlarge and divide mitotically while the oosphere is being differentiated.

2. The oosphere is differentiated through a massing of the cytoplasm of the oogonium. By this process the nuclei, usually in stages of mitosis, together with the vacuoles, are expelled from the central region, and there results a dense and coarsely vacuolate periplasm. This condition occurs when the antheridial tube is very short.

3. There is a stage called zonation in which the nuclei, usually in metaphase, are lined up around the ooplasm, some of the spindles lying across the definite boundary that separates ooplasm from periplasm. In the telophase of this mitosis about fifty daughter nuclei are found in the ooplasm.

4. The antheridium contains at first about thirty-five nuclei which divide twice mitotically, and simultaneously with the division in the oogonium and oosphere.

5. Previous to the entrance of the antheridial tube a papilla is found projecting from the oogonium into the antheridium.

6. The antheridial tube penetrates slowly, reaching the ooplasm at the time of zonation, later entering the oosphere and appearing as a conspicuously multinucleate structure. When it opens there are discharged about one hundred male nuclei which fuse with the female nuclei in pairs.

7. The sexual nuclei differ in form; the sperm being elongated and the egg spherical.

8. A peculiar central body, the cœnocentrum, develops as the oosphere matures and disappears before fertilization. Its function is unknown. There is some evidence of its being a dynamic center of the compound oosphere.

9. The mitoses are alike in the oogonium and antheridium. The spindle is intra-nuclear and there are no extra-nuclear radiations. The centrosomes are very prominent at metaphase, and are intra-nuclear. They could not be distinguished in the

resting nucleus. The nuclear membrane persists until after metaphase and the nucleolus is present throughout the division.

10. The primitive wall of the oospore first appears when the antheridial tube opens. Later the epispore is laid down upon it by the periplasm.

11. Two endospores are formed by the ooplasm after the development in the vacuoles of a peculiar substance which disappears as the endospores reach maturity.

12. After the complete encasement of the oospore it becomes rapidly filled with food-stuffs. A large central oil-like drop is present during the winter condition,

13. The fusion nuclei pass the winter in the resting condition without further perceptible change.

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EXPLANATION OF PLATES XI–XV.

All figures are from material killed in chrom-acetic acid and stained with Flemming's triple stain, unless otherwise indicated. The figures were sketched with an Abbé camera, using the following combinations of lenses: Zeiss 2^{mm} 1.30, with compensating ocular 18 and 12; also Bausch & Lomb $\frac{1}{12}$, ocular no. 4. These combinations give respectively the following magnifications when projected to the table level: 3300, 2200, and 1500 diameters. *Plate XI* was not reduced in reproduction. All other plates are three fourths of the original scale.

PLATE XI.

All drawings represent a magnification of 3300 diameters, and were studied with Zeiss lenses.

FIG. 1. Resting nucleus in mycelium, linin faint, nucleolus prominent.

FIG. 2. Nucleus in flowing cytoplasm near entrance of oogonium.

FIG. 3. Nucleus in antheridium, spirem stage, membrane faint.

FIG. 4. Prophase: small drops accumulated on the linin network, the itself thinner, nucleus becoming spindle-shaped, no centrosome visible.

FIG. 5. A stage somewhat later, linin threads have almost entirely disappeared, chromatin grains scattered through nucleus, nucleolus to the right, the remains of a linin strand to the left.

FIG. 6. Nucleus more spindle-shaped, globules arranged in the equatorial plate, at the poles round bodies about equal to the chromatin dots in the spindle fibers, the definite line bounding the whole of the nuclear membrane.

FIG. 7. Similar to *fig. 5*. The longitudinal lines probably chromatin which has not reached the equator, nucleolus at the left. This nucleus was crowded by a mass of others, hence its short form.

FIG 8. Similar to *fig. 7*. Fibers visible toward the poles. This spindle was formed in one of the strands supporting the forming oogonium, and was consequently much elongated by tension. It is from the oogonium shown in *fig. 62*.

FIG. 9. Chromosomes at the equator, spindle fibers very apparent at the poles, but not visible at the equator, nucleolus to left, membrane intact and inclosing the spindle.

FIG. 10. From oogonium shown in *fig. 60*. Cross section of a spindle, twelve chromosomes apparent (stained by hæmatoxylin from Flemming's material).

FIG. 11. Spindle mature, chromosomes closely grouped at the equator, centrosomes prominent, spindle brilliant and clear, nuclear membrane present but poorly stained (*fig 13*).

FIG. 12. Chromosomes splitting, membrane visible with nucleolus enclosed.

FIG. 13. Nucleolus large, nuclear membrane very definite, daughter chromosomes ready to separate. Only those in the highest focus are shown, several others being found at a deeper focus. The stain was particularly to show the membrane, and was not suitable for centrosomes.

FIG. 14. Anaphase: chromosomes separating, nucleus lying about midway, centrosomes still visible, whole nucleus staining dark and membrane indistinguishable from spindle fibers.

FIG. 15. Chromosomes nearing the poles, centrosomes not distinguished from them, nucleolus midway, slight traces of spindle fibers stretching across the middle space, cytoplasm in the ends of the nucleus stains darker than that of the central area.

FIG. 16. Whole spindle elongated, chromosomes massing together at the poles.

FIG. 17. Similar to *fig, 16*, but in a crowded position. Compare with *fig. 7*.

FIGS. 18 and 19 are from the periplasm of an oogonium of about the age shown in *fig. 67*, slightly younger than shown in *fig. 68*.

FIG. 18. Spindle fibers collapsing in the middle leading to the separation of the daughter nuclei, the fibers constituting the origin of the membrane of the daughter nucleus.

FIG. 19. A young daughter nucleus, nucleolus, membrane, and chromosomes.

FIG. 20. Same as *fig. 19* in resting stage, nucleolus prominent, linin faint.

FIGS. 21 and 22 are from the same oosphere, and in the condition shown in *figs. 68-70*.

FIG. 21. Same as *fig. 20* but passing into the spirem stage.

FIG. 22. Nucleus elongating preparatory to division. Compare *fig. 4*.

FIG. 23. Breaking of skein into chromosomes, centrosomes apparent.

FIG. 24. Spindle forming inside of nuclear membrane, nucleolus lying outside of the spindle.

FIG. 25. From an oospore of the condition shown in *fig. 70*. Spindle lying completely inside of the nuclear membrane, chromosomes grouping at the equator, and centrosomes well defined.

FIG. 26. Metaphase: chromosomes splitting, membrane, centrosomes, and fibers clear. Fibers apparently of about the same number as the chromosomes.

FIG. 27. Daughter chromosomes ready to leave the equator.

FIG. 28. Anaphase: chromosomes well separated, centrosomes visible, spindle fibers crossing the middle space, chromosomes six in number. From same oospore as *figs 24* and *27*. Compare with *fig. 16*.

FIG. 29. Later anaphase: chromosomes near the poles, area in which they rest darker stained than central portion.

FIG. 30. Similar to *fig. 18*. Spindle fibers collapsed and daughter nuclei ready to separate. From same oospore as *fig. 29*.

FIG. 31. A male nucleus from the entrance of antheridial tube. This nucleus is the same as the one marked X in *fig. 73*.

FIG. 32. A nucleus (sperm) in the tip of the same tube that contained that shown in *fig. 31*, also marked X. Wall of the antheridial tube, dense cytoplasm surrounding nuclei, sperm with nucleolus and mass of chromatin at anterior end.

FIG. 33. Tube open, elongated and pointed sperms escaping, showing a very faint linin network, one female nucleus shown. This is part of the drawing shown in *fig. 85*.

FIG. 34. A sperm approaching a female nucleus, linin more prominent than in *fig. 33*.

FIG. 35. A sperm in contact with a female nucleus, becoming more nearly rounded, and its linin still more prominent.

FIGS. 36 to 40. Various stages of fusion, from oospores of the general appearance represented in *fig. 90*. During fusion the whole nucleus becomes more darkly stained with gentian-violet.

FIG. 41. Cœnocentrum with globule at its center, female nucleus near by, many vacuoles of the oosphere near the margin.

PLATE XII.

Magnification in all figures 1500 diameters.

FIG. 42. A portion of mycelium, showing nuclei with prominent nucleoli.

FIG. 43. Nuclei and cytoplasm flowing into a developing oogonium, nuclei and vacuoles elongated and angular, nuclei too darkly stained to show structure.

FIG. 44. The septum below the oogonium, a portion of mycelium and posterior end of oögonium, oogonial nuclei assuming the spirem stage, mycelial nuclei still in resting condition with prominent nucleoli but enlarged.

FIG. 45. Oogonium and antheridium, nuclei of both in the spirem stage.

FIG. 46. Shows location of oil drops in an oogonium of the condition presented in *fig. 45*. They were all drawn at one focus; a slight change of focus would have brought vastly more into view.

FIG. 47. Showing adhesion of the oogonial *Hautschicht* to its wall in the neighborhood of the antheridium, nuclei overstained.

FIG. 48. Adhesion as in *fig. 47*. Oogonial wall partly and irregularly corroded away on the side toward the oogonium.

FIGS. 49–55. Stages in the perforation of the wall preparatory to the entrance of the antheridial tube.

FIG. 49. Optical section of papilla, wall partly corroded away and bulging toward the antheridium. The dense protoplasm represented in black was stained a deep red by the safranin.

FIG. 50. Sectional view of a condition slightly older than *fig. 49*. The shaded portion of the separating wall took the stain differently from the rest and was apparently in the last stages of dissolution.

FIG. 51. A papilla of different shape in a stage similar to the last.

FIG. 52. Optical section, stain as in *fig. 49*.

FIG. 53. A papilla becoming bubble-like, walls very thin, slightly shrunken as though due to imperfect killing, watery vacuoles apparent, their contents staining homogeneously with the gentian violet.

FIG. 54. Bubble-like papilla expanding irregularly in all directions, contents highly vacuolate, wall extremely thin.

FIG. 55. Wall almost perforated, but showing no marked bulging in either direction, *o*, oogonial, *a*, antheridial side.

FIGS. 56, 57. Young antheridial tubes with a cellulose wall and surrounded by a dense sheath of the protoplasm of the oogonium.

FIG. 58. Protoplasm beginning to collect in masses, nuclei approaching metaphase, some shown in polar view (preparation from material fixed in Flemming's agent and stained by hæmatoxylin).

Fig. 59. An intercalary oogonium, slightly older condition than *fig. 58*, lasm distinctly in masses, nuclei in early prophase, antheridial tube just entering bearing no nuclei, a haustorium at extreme right.

PLATE XIII.

Magnification in *figs. 60, 61, 62, 63, 64, 65, 68, 69, 70, 72*, 1500 diameters; in *figs. 66, 67, 71*, 3300 diameters; in *fig. 73*, 2200 diameters.

Fig. 60. Nuclei in metaphase, protoplasm massed in a few centers, es very clear and brilliant (stain hematoxylin from material fixed in Flemming's agent).

Fig. 61. Nuclei in late prophase, the protoplasmic masses coalesced to ne, vacuoles mark the juncture last made, a few nuclei not yet floated out.

Fig. 62. Nuclei approaching metaphase, spindles much elongated, all not extruded from the central region, mitosis in the antheridium, antheridial tube shown at its typical position for this stage.

Fig. 63. Diagram of disposition of oil drops in an oogonium of the condition shown in *fig. 65* (zonation).

Fig. 64. Nuclei nearly at metaphase, zonation almost complete, oil-like the right in ooplasm, antheridial tube present showing no nuclei.

Fig. 65. Zonation: nuclei near metaphase, ooplasm sharply differentiated, spindles lying directly across the boundary between ooplasm and peri- (stained by hæmatoxylin from Flemming material). See also *fig. 64*.

Fig. 66. Shows spindle in metaphase lying across the film between asm and periplasm.

Fig. 67. Nucleus in anaphase directly across the boundary film of the asm, also half of a late anaphase cut diagonally.

Figs. 68, 69. Consecutive sections of an oogonium just after the division nuclei, showing the position and shape of the antheridial tube. *Fig.* shows the cœnocentrum which had just passed its maximum development *fig. 71*).

Fig. 70. Antheridial tube and differentiating oosphere, oosphere nuclei tosis. *Fig. 74* was taken from an adjacent section of the same oogonium shows the cœnocentrum.

Fig. 71. Completely developed cœnocentrum, the central globule surded by three regions of differentiated protoplasm. From a stage of zonahere the nuclei were in anaphase (as in *fig. 67*) and the daughter nuclei about to pass into the ooplasm.

Fig. 72. Diagram of distribution of oil at the stage shown in *fig. 70*.

Fig. 73. Antheridium and tube of the age shown in *fig. 70*, showing nucleate contents; *l*, film; ♀, one of the female nuclei; *p*, periplasm; nuclei, represented also in *Plate XI*, *figs. 31, 32*.

PLATE XIV.

Magnification in *figs. 74, 80, 82, 87, 88*, 1500 diameters; *fig. 76*, [illegible] diameters; *figs. 75, 77, 78, 84, 85, 86*, 3300 diameters.

FIG. 74. Cœnocentrum and differentiated oosphere with dividing nuclei. The broken empty portion is where the protoplasm shrunk away from the antheridial tube. Section adjacent to that shown in *fig. 70*.

FIG. 75. Cœnocentrum, in an oogonium, at anaphase period of zonation, consisting of three small globules evidently fusing, surrounded by a region of denser protoplasm, granules resembling small oil drops scattered through the ooplasm.

FIG. 76. Similar to *fig. 73* but somewhat older, antheridial tube showing many nuclei, slightly torn near tip, antheridium becoming vacuolate; *p*, periplasm; *b*, film; ♀, female nucleus.

FIG. 76 *a*. Portion of the tip of the same tube, found in an adjacent section.

FIG. 77. View of the end of an unopened antheridial tube, wall not apparent, sperms very numerous and crowded, each showing a dark nucleolus; ooplasm slightly shrunken away from the tube.

FIG. 78. Section near the base of the same antheridial tube, showing very thick wall in contrast with the extremely thin film covering the apex.

FIG. 79. Diagram to show position of preceding sections. *Fig. 77* was from a section just above the line *aa*; *fig. 78* from one just below the line *bb*. One intermediate section was not drawn.

FIGS. 80, 80*a*. Adjacent and approximately longitudinal sections of the opening antheridial tube of the same oogonium, showing the primitive wall and the increased vacuolation of the ooplasm which frequently shrinks away from the primitive wall.

FIG. 81. Diagram of the distribution of oil in an oogonium of the age shown in *fig. 80*.

FIG. 82. Antheridial tube discharging sperms, antheridium shown above, primitive wall very young, ooplasm becoming vacuolate, antheridial tube cut slightly oblique so that its base is in an adjacent section.

FIG. 83. Diagram to show relation of sections presented in *figs. 84, 85, 86*: *fig. 84* was cut from above the line *aa*, and tangential to the tip of the opening tube; *fig. 85* from between the lines; *fig. 86* from just below the line *bb*.

FIG. 84 (see *fig. 83*). Sperm nuclei leaving the antheridial tube and approaching the female nuclei.

FIG. 85 (see *fig. 83*). Mass of sperms escaping from tube. The tube may be traced to the left as a mass of darkly stained structureless protoplasm

The sperms in a mass appear dark but individually are hyaline except at anterior end which bears the nucleolus. See also *fig. 33*, *Plate XI.*

FIG. 86 (see *fig 83*). The base of the antheridial tube filled with dark staining cytoplasm and few nuclei.

FIG. 87. Just after discharge from antheridial tube, periplasm as in *fig.* [illegible], several small masses of nuclei apparently both male and female surrounded by denser cytoplasm (these masses seem to arise by the breaking apart of the contents of the antheridial tube and soon disappear), primitive wall well developed, antheridium above, ooplasm coarsely and irregularly vacuolate.

FIG. 88. Nuclei fusing in pairs, primitive wall distinctly thickened. See *Plate XI, figs. 36–40.*

PLATE XV.

All figures magnified 1500 diameters.

FIG. 89. Protoplasm collecting in dense network toward the center of the oospore, leaving light peripheral strands where the endospore is soon to appear.

FIG. 90. Shows the remains of the antheridial tube in the periplasm, with a trace of its former presence in the ooplasm. The oospore is of the age shown in *fig. 88.*

FIG. 91. Primitive wall mature, exospore forming, now consisting of a [illegible] semi-transparent mass with imbedded disks which are to form the ridges, vacuoles in oospore filled with gelatinous substance, nuclei of protoplasm overstained.

FIG. 92. Somewhat younger than the last figure, showing structure of exospore more clearly, presents edge view and also a fragment bent back and giving a surface view, gelatinous substance in vacuoles.

FIG. 93. Exospore nearly formed, primary endospore complete, vacuoles containing gelatinous substance.

FIG. 94. Rudimentary secondary endospore, gelatinous substance in vacuoles.

FIG. 95. Oil forming in oospore.

FIG. 96. Spore walls complete, including double endospore, vacuoles with no gelatinous substance, oil accumulating in large drops on the endospore walls.

FIG. 97. Winter conditions of exospore, the ridges higher than in previous figures, large central oil-like mass, nuclei in sporoplasm. Section not directly through middle so that the endospore appears thicker than it really is.

FIG. 98. Exterior of ripe oospore.

NOTES ON THE DEVELOPMENT OF THE HOLDFASTS OF CERTAIN FLORIDEÆ.

CARRIE M. DERICK.

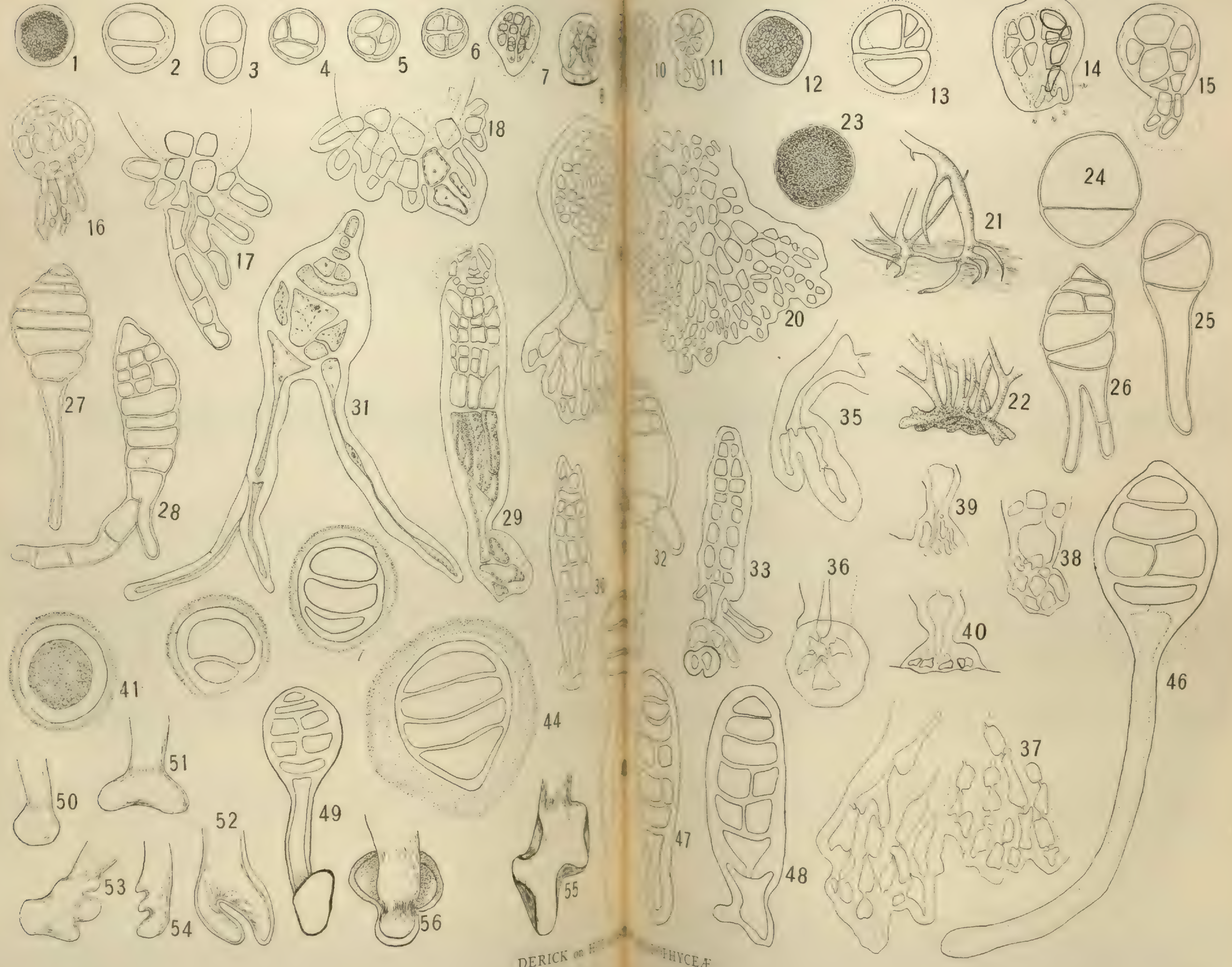
(WITH PLATES XXI–XXIII AND FIVE TEXT-FIGURES)

WITH the exception of passing references in various works two articles, the one by Borge (1), the other by Strömfelt (8), include, I believe, all that has been written upon the holdfasts of the algæ. The former deals with a few members of the Chlorophyceæ; the latter is very comprehensive, but it is without illustrations and gives no specific details. Therefore, the study of the development of the holdfasts of some nearly related species of the Rhodophyceæ has seemed advisable.

The observations described in this paper were made at the Marine Biological Laboratory, Woods Hole, Mass., during the summers of 1896 and 1897, and the work was finished in the Botanical Laboratory of McGill College.

Cultures of the spores of several species were made under various conditions. Ordinary glass object-slides were placed in flat porcelain dishes, either white or painted black. The vessels were filled with filtered sea-water and in them were laid plants bearing ripe spores. The spores usually sowed themselves in twenty-four hours, in which case the plants were removed. Some of the dishes were fed by a gentle stream of running water; others were disturbed only three times a day, when the water was drawn off by a siphon and replaced by filtered sea-water. The latter method proved much the better of the two.

The color of the background had no effect upon the development of the spores, nor were the plantlets heliotropic. Cultures kept in a shaded place flourished best, even a short exposure to direct sunlight killing plantlets. It is to be regretted that no record of variations in the temperature and the density of the water was kept. Oltmanns (6) has conclusively shown that such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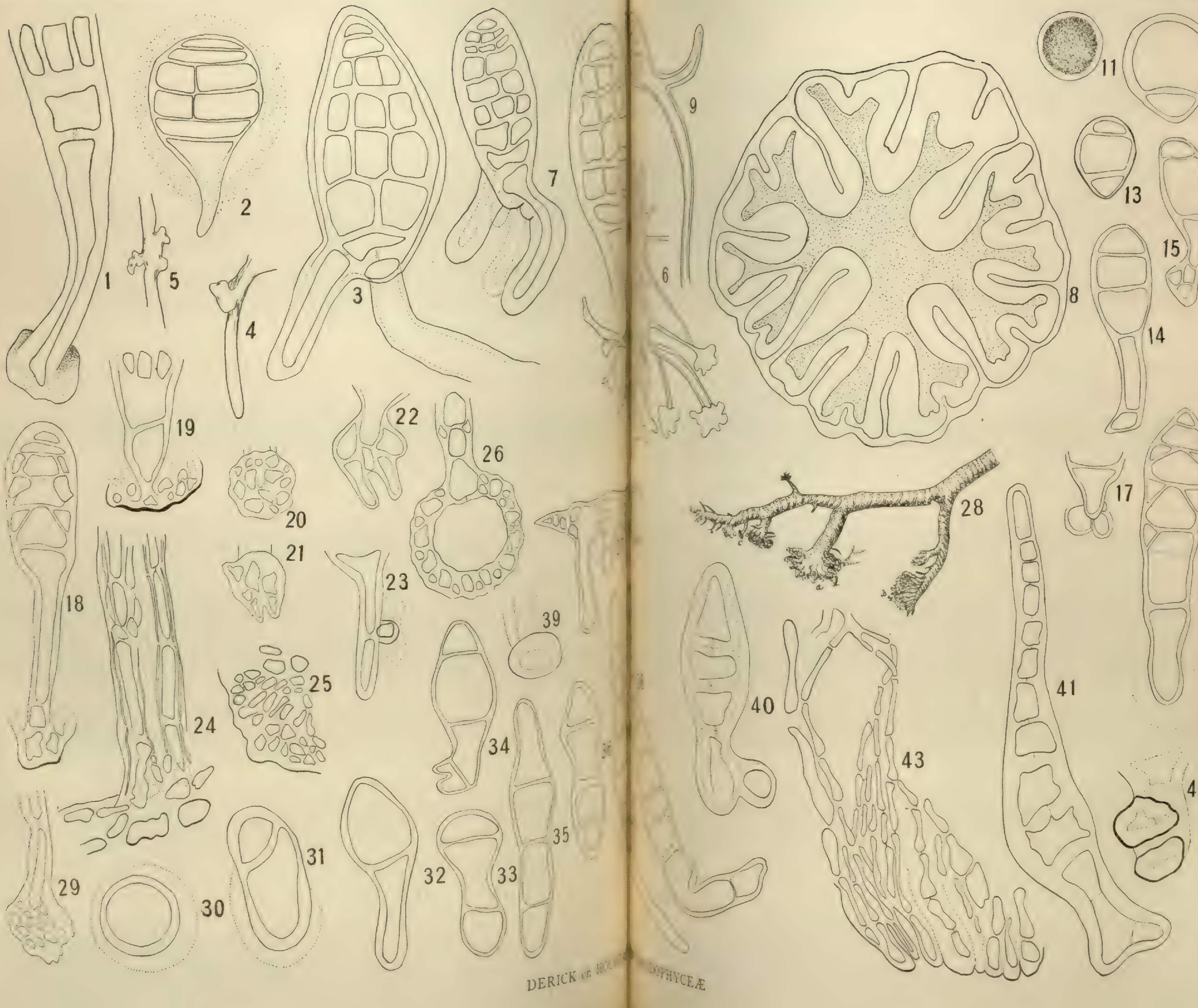
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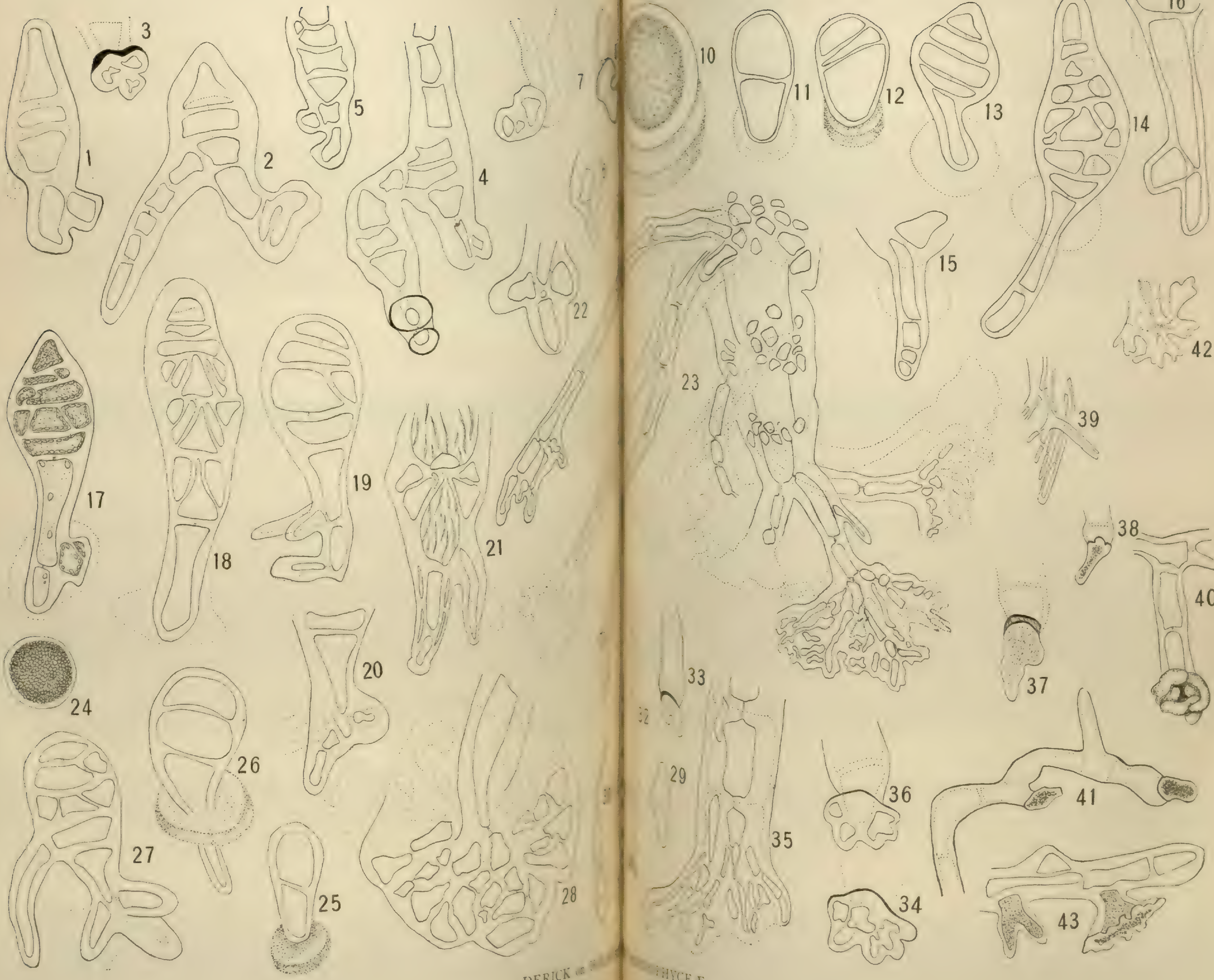
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DERICK on HYCEÆ

variations are most important factors in the life and distribution of marine algæ, but a comparison of many cultures of the same species indicates that differences in the conditions which prevailed in the laboratory affected neither the form nor the order of development of the plantlet. It was difficult to keep the young plants healthy for more than three or four weeks; and during a few days of intense heat, in August 1896, all ceased to grow, were attacked by bacteria, and finally died.

Both carpospores and tetraspores were germinated and most closely resembled one another in their development. In some cases, however, tetraspores attached themselves to the substratum less readily than carpospores. This is regarded by Brannon (2) as an adaptation to the immediate distribution of the species.

Every day, slides upon which spores were growing were examined and drawings of living plantlets were made. Permanent mounts for comparative study were prepared at regular intervals and mature holdfasts were preserved in alcohol or in 5 per cent. formalin in sea water.

The species selected for investigation belonged to the Rhodymeniales, with the exception of one member of the Rhodophyllidaceæ, *Rhabdonia tenera* J. Ag. The carpospores of this alga attach themselves firmly to the slides in nine hours; a very delicate outer layer, probably of mucilage, may be observed, but can hardly be distinguished from the cell-wall (*Pl. XXI, fig. 1*). In a few cases, an irregular layer of a coarsely granular substance surrounded the plantlets, and in one, a thick mucilaginous disk was developed at the base; these appearances were most exceptional and were doubtless due to slightly abnormal conditions (*Pl. XXI, figs. 7, 8*). After attaching themselves, the spores immediately enter upon a segmentation stage, and within twenty-two hours two divisions are made. The first separates the spore by means of a vertical wall into two equal cells (*Pl. XXI, figs. 2, 3*). Three and four-celled stages result from the successive division of the two primary cells in vertical planes at right angles to the first wall; other cells are cut off from these by oblique walls, and thus an irregular spherical mass is formed.

In about thirteen days differentiation begins. Four basal cells elongate to form the primary root-cells, the position of which bears no definite relation to the direction of the supply of light (*Pl. XXI, figs. 7, 8*). Occasionally only one or two primary rhizoids are thus formed (*Pl. XXI, figs. 9, 10, 11*). Unfortunately, the plantlets died at this stage, and only by analogy could the subsequent history of the holdfasts be determined. However, the close resemblance between the primary root-cells and mature holdfasts of Rhabdonia and of the two species to be considered next justifies the assumption that the intermediate stages closely agree. It is interesting to note that, according to Osterhout (7), the earliest divisions in the tetraspores which give rise to proliferations do not succeed one another in the definite order just described, but the spores divide "by means of oblique walls which do not occur in regular succession."

Very similar to that of Rhabdonia is the early history of *Lomentaria uncinata* Menegh. and of *Champia parvula* Harv. The carpospores of Lomentaria, after secreting a wall, attach themselves to the substratum by a very thin mucilaginous secretion (*Pl. XXI, fig. 12*), which sometimes persists after the first rhizoids have been formed (*Pl. XXI, fig. 15*). The segmentation proceeds rapidly, but primary root-cells do not arise until a later date than in Rhabdonia (*Pl. XXI, figs. 13, 14*). The four basal root-cells, dividing by horizontal walls, become short, closely-appressed filaments, which soon branch pseudo-dichotomously (*Pl. XXI, figs. 15, 16*) and form a discoid holdfast. Strömfelt (8) regards the branching of the rhizoidal filaments as dichotomous and says that the increase in circumference of the mature holdfast is due almost entirely to marginal growth. Vegetative reproduction may take place not only by the mechanical separation of the various upright branches, which spring adventitiously from the surface of the holdfast (*Pl. XXI, fig. 22*), but by means of stolons, which are sent out in various directions and form secondary holdfasts (*Pl. XXI, fig. 21*).

Davis's account (4) of the plantlets of *Champia parvula* developing under natural conditions, accurately describes those grown in the laboratory. A somewhat spherical mass composed of sixteen cells results from the segmentation of the spore, then the first indication of a permanent holdfast appears as a slight projection of each of the four basal cells. The primary root-cells, dividing at right angles to their longest axis, form two-celled filaments, which branch monopodially and give rise to a broad spreading holdfast. In section the mature holdfast appears parenchymatous (*Pl. XXI, figs. 17, 18*), but it is often possible to distinguish the component filaments (*Pl. XXI, fig. 20*). Exceptional plantlets produce one instead of four primary root-cells, but the later stages conform to the type (*Pl. XXI, fig. 19*). In all cases the cells of the holdfast are paler than those of the frond, and the chromatophores of young specimens are in close contact with the walls.

From the foregoing it will be seen that Rhabdonia, Lomentaria, and Champia agree (*a*) in passing through a segmentation stage, resulting in a somewhat spherical mass of cells, (*b*) in the elongation of four basal cells, and (*c*) in the subsequent development of four primary rhizoids, which branch repeatedly and finally form a large discoid holdfast, composed of pseudoparenchymatous tissue.

In marked contrast are several members of the Rhodomelaceæ. The spores of *Chondria tenuissima* (Good. et Wood.) C. Ag. and of *Chondria dasyphila* Ag. germinate very readily. The early divisions of the spores and the history of the development of the rhizoids are alike in the two species. The spores, which are large and have coarsely granular contents, quickly and firmly attach themselves to the substratum, doubtless by means of a thin and uniform layer of mucilage, though no secretion distinct from the cell-wall is perceptible (*Pl. XXI, fig. 23*). They soon divide into two unequal cells separated by a slightly concave wall; divisions parallel to the first follow (*Pl. XXI, fig. 24*), and the basal cell of the resulting filament elongates into the primary rhizoid (*Pl. XXI, figs. 25, 27*). Sometimes the basal cell seems to branch dichotomously; but, as the branches do not arise simultaneously nor are they separated by a wall parallel to the longer axis of the basal cell, it is evident that the branching of the filaments is strictly monopodial (*Pl. XXI, figs.* 26, 32, 35). The upper cells of the plantlet soon cut off pericentral cells and at the same period the first rhizoid divides repeatedly so as to form a multicellular, monosiphonous filament (*Pl. XXI, figs.* 28, 35). Secondary rhizoids are next developed, either as outgrowths from the primary root-cell or from a pericentral cell immediately above the basal cell (*Pl. XXI, fig.* 31). Long slender rhizoids may be formed before a clasping-disk arises, but, both in plantlets developing in the laboratory (*Pl. XXI, figs.* 29, 33, 36) and in those growing in a state of nature (*Pl. XXI, figs.* 30, 38, 39, 40), the primary rhizoid generally remains very short and by means of oblique walls produces discoid holdfasts at the tip. The size and efficiency of this holdfast are increased by means of free secondary rhizoids (*Pl. XXI, fig.* 33) or by intracuticular filaments (*Pl. XXI, fig.* 34), both of which have their origin in pericentral cells near the base of the plantlet. The secondary rhizoids develop in the same manner as the primary and combine with them to form a large, irregular, discoid holdfast, in which it is possible to distinguish few of the component filaments (*Pl. XXI, fig.* 37). The mature holdfast, though adhering closely to its host, does not penetrate it, but a cutinization of the cortical layers often results from the contact. As in Lomentaria, etc., the large holdfast gives rise to several adventitious branches; and great powers of vegetative reproduction are implied by the stores of floridean starch with which the cells are often charged. The chromatophores are closely aggregated in the peripheral protoplasm, especially next to the inner radial and the lateral walls, and often indicate the plane in which a wall will soon be formed. They are large and discoid, thus resembling those of the cortical cells of the frond rather than the anastomosing filamentous chromatophores of the central cells.

Very different from the short, multicellular, primary rhizoids of the Chondrieæ are the primary holdfasts of the Polysiphonieæ. The form taken as a type of the latter group is

Polysiphonia violacea (Roth) Grev. In a few hours after they are sown, both carpospores and tetraspores attach themselves to the substratum by a coarsely granular, mucilaginous secretion, which completely covers the spore (*Pl. XXI, fig. 41*). In optical section this envelope appears densely granular at the margin, while a clear amorphous area intervenes between the outer layer and the developing plantlet (*Pl. XXI, fig. 44*). The spore divides into two unequal cells, of which the smaller soon becomes slightly pointed and finally grows into the primary root-cell (*Pl. XXI, fig. 42*). Divisions parallel to the first ensue (*Pl. XXI, figs. 43–45*), and the basal-cell elongates so as to form the first rhizoid piercing the mucilaginous sheath, which finally disintegrates and disappears. A six-celled stage is often reached before divisions in planes at an angle to the first occur. Generally, segmentation continues for some time and the siphons are clearly marked off from the central axis before a second rhizoid arises. As in Chondria, this has its origin in an unsegmented cell adjacent to the primary root-cell. The former differs from the latter only in its brighter color and denser cell-contents; and, remaining undivided, it forms a component part of the holdfast (*Pl. XXI, fig. 50*). The protoplasmic connections between these cells are obvious, but they are difficult to trace between the other cells of the plantlet (*Pl. XXI, figs. 45, 47*). When three or four weeks old the young plant develops several rhizoids springing either from the primary or secondary root-cell (*Pl. XXII, fig. 7*). Occasionally, however, the segmentation stage of the spore ends and the growth of the frond begins before multiplication of the rhizoids takes place (*Pl. XXII, fig. 10*). At the base of the frond other rhizoids are sent out by corticating cells and are separated from these by a wall, a protoplasmic connection being maintained. No intracuticular filaments are developed and the independence of the rhizoidal constituents of the mature holdfast is practically preserved (*Pl. XXII, fig. 6*). In addition to the rhizoids near the base of the plant, any corticating cell of a procumbent branch may produce a secondary holdfast and thus assist in the extension of the colony. As Strömfelt (8) noted, all the rhizoids are unicellular and unbranched; and, although an apparent tendency to branch may be observed occasionally, the lobes are not separated from the main portion by a wall, even the protoplasmic contents being undivided (*Pl. XXII, fig. 9*). All the rhizoids eventually develop terminal clasping-disks. The first indication of such a structure may appear in plantlets four days old, but, as a rule, the primary rhizoids do not undergo modification until several days later. The disks begin as a simple enlargement of the tip of the rhizoid (*Pl. XXI, figs. 48–50; XXII, fig. 1*), become deeply lobed, and assume a very irregular outline (*Pl. XXI, figs. 52–56*). The cell-contents of the rhizoid extend into the lobes, but no division takes place (*Pl. XXII, fig. 8*). Great variations in the length attained by the primary rhizoids occur both in plants grown under natural conditions and in laboratory cultures (*Pl. XXI, figs. 46, 48; XXII, fig. 1*). The cause of such variations has not been determined, but it is probable that contact irritation may be the most important factor in the formation of the disks. This view is supported by the fact that disks are sometimes produced on the sides of rhizoids when these come in contact with a firm substance (*Pl. XXII, figs. 4, 5*). The length of the secondary rhizoids depends upon the distance of the parent cells from the substratum, and as soon as contact is established broad clasping-disks are formed, which mechanically cohering with one another and with the primary disk produce a very strong holdfast. The rhizoids are paler in color than the rest of the plantlet, having less dense contents and fewer chromatophores.

The rhizoids of *Polysiphonia violacea* never penetrate the host-plant; but at the point of contact the surface of the latter is often dark brown and cutinized, while the outer cortical cells are destitute of chromatophores. But incipient parasitism occurs in *Polysiphonia fastigiata* Grev., growing on *Ascophyllum nodosum* Stack. Gibson (5), in writing of the histology of this species, noticed that "the attachment of the epiphyte to Ascophyllum is very intimate. Root-filaments given off from the base of the frond penetrate deeply into the tissue of the host, and wander among the cortical cells and medullary hyphæ. The root-filaments have very thick cell-walls and central cells only, these being much elongated." The ends of the rhizoids are swollen and in close contact with the cells of the host (*text figs. 4, 5*, p. [illegible]), but no haustoria penetrate the walls of the latter. In one instance a unique variation occurred. A few intracuticular filaments, descending from the corticating cells of the Polysiphonia, ran parallel to the main axis of the rhizoid throughout its length. The host suffers no serious injury, only a depression and cutinization of the surface with a very slight disorganization of the cortical cells at the point of penetration. Though the association of the two plants does not justify the assumption of complete parasitism, the symbiotic relation existing between them is much more intimate than that observed between Ascophyllum and any of the truly epiphytic algæ. According to Brebner (**3**), a similar relation exists between *Dumontia filiformis* and its host, *Fucus serratus*.

Dasya elegans (Martens) C. Ag. was the third species of the Rhodomelaceæ examined. The spores attach themselves by a mucilaginous secretion much less definite in form and less persistent than in *Polysiphonia violacea* (*Pl. XXII, fig. 30*). The spore elongates before the division takes place, and in many instances the very young plantlet assumes an hour-glass shape, occasionally with a delicate mucilaginous secretion at either end. The first walls are parallel to one another, forming a long filamentous body, of which one terminal cell becomes the apical cell of the frond, the other the primary root-cell (*Pl. XXII, figs. 31, 32, 33, 35, 36*). Twelve or more parallel divisions may occur before the basal cell elongates and forms a rhizoid terminating in a disk; but such a modification may appear at an earlier period (*Pl. XXII, fig. 34*). The multicellular disks, which arise sooner or later, are like those of Chondria. The root-cell or the end of a rhizoid broadens and becomes slightly lobed; oblique walls cut off the lower corners of the cell; division is continued and a multicellular disk with a mucilaginous margin results (*Pl. XXII, figs. 39–41; XXIII, figs. 2, 3, 7, 8, 9*). The primary root-cell often branches, each portion giving rise to a disk (*Pl. XXII, fig. 42; XXIII, fig. 4*). Although the primary rhizoid usually remains short, in some instances it attains considerable length before undergoing division or forming a disk (*Pl. XXII, figs. 37, 38*). While these changes are taking place, the cell adjacent to the basal cell sends out rhizoids similar to those arising from the primary root-cell (*Pl. XXIII, figs. 1, 5, 6*). These various root-filaments combine to form the primary holdfast, which is afterwards strengthened by multicellular branching rhizoids, springing from the basal corticating cells of the frond. The course of the filaments may be traced for some distance in the holdfast, but it is difficult to distinguish between those cells which have their origin in the primary disk and those which are derived from the corticating filaments. The difficulty in determining the relationship of the parts is increased by secondary lateral connections, which are developed between the corticating cells (*Pl. XXII, fig. 43*). The marginal cells of the mature holdfast are larger and broader in proportion to their length than the corticating cells of the frond, and have denser cell contents, but the chromatophores of both are separate disks, while those of the central siphon are the anastomosing filaments characteristic of many of the Rhodomelaceæ. A creeping tendency may be exhibited at an early age, very young plantlets sometimes developing two distinct holdfasts of almost equal importance (*Pl. XXIII, fig. 1*). As in the other species described, many branches arise from the massive rounded holdfast, probably springing adventitiously from the surface of the latter.

A comparison of the three species of the Rhodomelaceæ described will show that they agree in forming a primary root-cell, which elongates into a rhizoid terminating in a clasping disk; and in developing secondary rhizoids, which are sent out by the root-cell, the cell adjacent to it, and the cortical cells at the base of the frond. But, while the rhizoids of Polysiphonia are unicellular, unbranched, and free, those of Dasya and Chondria are multicellular, branched, and aggregated into a compact cell-mass, which in section resembles parenchymatous tissue.

The species remaining for consideration belong to the Ceramiaceæ. Cultures of *Spermothamnion Turneri* Aresch. were unsuccessful, only one healthy plantlet having been obtained. It developed two small disks, the one a terminal primary holdfast, the other a secondary structure arising from one of the middle cells of the filament (*Pl. XXIII, fig. 41*). As Strömfelt has pointed out, the mature rhizoids are short, unicellular, unbranched organs, terminating in a lobed disk in which delicate threads of protoplasm can be traced (*Pl. XXIII, figs. 42, 43*). The rhizoids are especially abundant near the base of the plants, but, as is well known, any cell of a procumbent branch may give rise to these simple bodies. Now and then, an ordinary branch of the frond produces at its apex a much-lobed twisted disk, which closely embraces a neighboring branch as a tendril would a support (*Pl. XXIII, fig. 40*).

Very few spores of *Griffithsia Bornetiana* Farlow germinated, and the resulting plantlets were short-lived. It is, therefore, impossible to describe each step in the development of the holdfast. Two unequal cells arise from the first division (*text fig. 1*), a monosiphonous filament composed of rather globose cells is formed, the basal cell of which elongates and becomes the root-cell (*text fig. 2*). The structure of mature holdfasts would lead one to suppose that the primary root-cell divides repeatedly, forming a broad spreading mass of large-celled pseudo-parenchymatous tissue. The holdfasts of a young plant attached to a small piece of bone (*text fig. 3*) and of older plants growing on *Zostera marina* L. differed only in size, that of the latter being very large with an abundance of adventitious branches arising from its margin. Strömfelt (8) places the genus Griffithsia with Ceramium and Callithamnion in a group distinguished by branching rhizoids; but, though many mature specimens of *Griffithsia Bornetiana* were examined, no such secondary formations were seen. The cells of the holdfasts are brilliantly colored and have very dense granular contents, probably due to a large supply of reserve food-material.

A closely allied genus, Callithamnion, differs greatly from

Griffithsia in the form and history of the holdfast. Though th spores of *Callithamnion Borreri* Ag. developed in the labora with difficulty, cultures sufficient to illustrate the order of dev opment succeeded. After attaching themselves to the slide b an almost imperceptible secretion, the spores elongate a become pointed at both ends. The first division is parallel the shorter axis (*Pl. XXIII, fig. 29*), and by subsequent partition

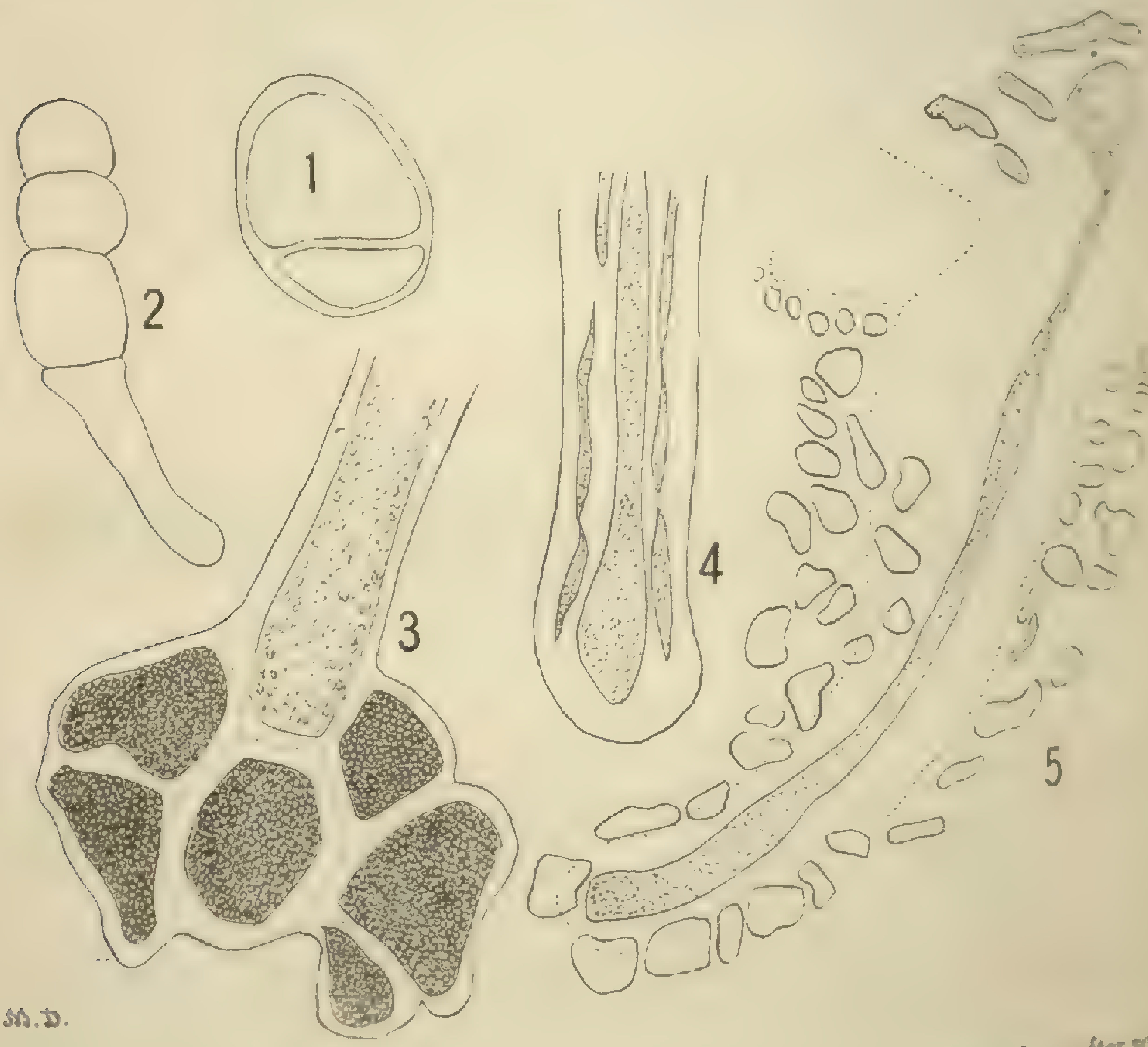

FIG. 1. *Griffiithsia Bornetiana* Farlow, germinating tetraspore 9 days after sow-ing. × 333.

FIG. 2. *G. Bornetiana*, plantlet 9 days old. × 213.

FIG. 3. *G. Bornetiana*, holdfast of small plant growing on bone. × 53.

FIGS. 4, 5. *Polysiphonia fastigiata* Greville, growing on *Ascophyllum nodos* Stack.; in *fig. 5* the cells of *P. fastigiata* are shaded in, those of Ascophyllum are merely outlined. × 213.

monosiphonous filament is formed. The smaller of the two ter-minal cells is the primary root-cell (*Pl. XXIII, fig. 30*). Judg from one set of cultures, the first cell of the holdfast, which a very thick mucilaginous wall, is cut off from the swo extremity of the root-cell and separated from it by a wall (*Pl. XXIII. figs. 31–33, 37, 38*). A multicellular holdfast results from the division and branching of the primary root-cell and of the first cell of the disk (*Pl. XXIII, figs. 34, 36*), and is further strengthened by a few corticating rhizoidal filaments. In mature *Callithamnion Baileyi* Harv. the intracuticular rhizoids are more numerous. They arise from the lower angles of the central cells or from the basal cells of the branches. Thence they descend through the walls of the monosiphonous frond to the holdfast, where they branch freely and spread out in various directions, forming, with the filaments arising from the cells of the primary disk, a flat circular holdfast (*Pl. XXIII, figs. 35, 39*).

The carpospores of *Spyridia filamentosa* (Wulf.) Harv. germinate very readily. The mucilaginous secretion, by which the spores are first fastened to the substratum, can hardly be distinguished from the wall of the spore (*Pl. XXII, fig. 11*). The spore first forms two unequal cells, the smaller of which becomes the primary root-cell, the larger divides by parallel walls so as to form a short filament (*Pl. XXII, figs. 12–14*). As a rule, no long primary rhizoid is formed, but occasional exceptions are found (*Pl. XXII, fig. 18*). Though variations may occur (*Pl. XXII, fig. 15*), the first corticating cells are usually cut off from the upper sides of the central cells before the formation of the discoidal holdfast begins (*Pl. XXII, fig. 16*). Sooner or later, however, the primary root-cell divides in several planes parallel to the longer axis of the spore, and thus produces a flat multicellular disk, the cells of which are separated by walls (*Pl. XXII, figs. 15, 17, 19, 21*). At first the primary disk is but slightly lobed, but the lobes soon give rise to short filamentous outgrowths, which branch pseudo-dichotomously (*Pl. XXII, figs. 21, 22*). Rarely a lateral lobe springs from the primary root-cell, which is then prolonged into a filament (*Pl. XXII, fig. 23*). The cells of the primary holdfast continue to divide, and with them are combined rhizoidal filaments, which have their origin in the corticating cells of the lower nodes of the frond and grow through the cell-walls of the substratum (*Pl. XXII, figs. 24, 25*). Free filamentous outgrowths of the larger cortical cells develop disks and enter into close mechanical union with the main holdfast, adding greatly to its efficiency. The secondary rhizoids thus formed are destitute of cortications (*Pl. XXII, fig. 29*) and are easily distinguished from those ordinary branches which, coming in contact with the substratum, develop holdfasts (*Pl. XXII, figs. 26, 28a*). Spyridia creeps not only by means of such branches but by the aid of masses of uncorticated hair-like rhizoids, which may be formed at any point (*Pl. XXII, fig. 28b*). One curious instance was noted of short rhizoidal outgrowths from each of the central cells of a trailing branch, the cortical cells being unmodified (*Pl. XXII, fig. 27*). The differences in the chromatophores are similar to those noted in the Rhodomelaceæ.

Ceramium differs in many respects from the other genera described. Both *Ceramium rubrum* (Huds.) C. Ag. and *C. strictum* Harv. were carefully studied and found to agree closely in so far as the early development of the plantlet and the formation of the rhizoid are concerned. Shortly after it is sown, the spore produces a cell-wall (*Pl. XXIII, fig. 24*) and a well-defined temporary holdfast. The latter is a granular disk of definite thickness, and mucilaginous in character, attached to the base of the spore (*Pl. XXIII, fig. 10*). The granules, embedded in a clear matrix, are often arranged in lines radiating from the axis to the rim of the disk (*Pl. XXIII, fig. 25*). The distances between the granules being least in vertical planes, the disk appears densest when viewed from the side. This peculiar body does not respond to the ordinary tests for cellulose, and is not dissolved after prolonged treatment with dilute potassium hydrate. It is affected neither by Hanstein's aniline blue nor safranin; but the granular portions stain deeply with hæmatoxylin and with Congo-red, and the whole with Bismarck-brown. The disk is, therefore, distinct from the cellulose wall of the spore and differs materially from ordinary vegetable mucilages, though it is probably closely allied to the latter. This temporary holdfast is not peculiar to plants existing in an unnatural environment, but has been found in very young plants growing on Chordaria. As the spore develops, the first rhizoid pierces the disk, which then becomes disintegrated and finally disappears (*Pl. XXIII, fig. 26*).

After attaching itself in the manner described, the spore elongates and divides several times in parallel planes at right angles to its longer axis (*Pl. XXIII, figs. 11–13*). The basal cell, growing rapidly, produces a multicellular rhizoid at an early stage; but large plantlets, which have already cut off corticating cells, occasionally show little or no tendency to form rhizoids (*Pl. XXIII, figs. 14, 15, 18*). The primary root-cell branches into several rhizoids, which are increased in number by outgrowths from the cell adjoining the first root-cell (*Pl. XXIII, figs. 17, 19, 27*). Still later, the cortications near the base of the plantlet develop multicellular branching rhizoids of great length. All remain free throughout the life of the plant, and both primary and secondary rhizoids branch monopodially near the tip, and thus give rise to large multicellular disks of irregular outline (*Pl. XXIII, figs. 16, 22, 23, 28*). These indented clasping-disks are closely crowded together, cohering so as to form a large rounded holdfast, in which the various elements may be clearly distinguished. As both of the species are upright in habit, no secondary holdfasts are developed at any point of the mature frond. As in several other genera, the chromotaphores of the plantlets and of the holdfasts resemble those of the corticating cells rather that those of the central axis, the former being disks, the latter irregular branching bands (*Pl. XXIII, figs. 17 and 21*).

It is evident, therefore, that the species of the Ceramiaceæ examined differ greatly both in the manner of development and in form of the holdfast, agreeing only in the production of one primary root-cell. *Spermothamnion Turneri* forms at various points short unicellular rhizoids with terminal disks, branching does not occur, and cortications are not developed. *Griffithsia Bornetiana* produces a large spreading holdfast composed entirely of pseudo-parenchymatous tissue arising from the primary root-cell. Callithamnion, Spyridia, and Ceramium have primary root-cells, from which spring rhizoids terminating in multicellular disks. Others originate in the cell adjacent to the basal cell and in the cortications. In addition the first two possess a strengthening mass of intracuticular root-fibers, but Ceramium is quite destitute of them.

Thus, while of some value in showing relationships, it will be seen that the chief interest in a comparative study of the developing spores and holdfasts of the Florideæ would be in variations dependent upon differences in light, temperature, or the density of the surrounding medium, and in adaptations to vegetative reproduction.

In closing, I would acknowledge my indebtedness to Dr. Setchell, who, in 1895, suggested the holdfasts of the Rhodophyceæ as a subject that would repay investigation; to the late Dr. Humphrey, under whose helpful and suggestive direction the work described in this paper was practically begun; and to Professor Penhallow for kind advice.

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EXPLANATION OF PLATES XXI–XXIII.

PLATE XXI.

FIG. 1. *Rhabdonia tenera* J. Ag., carpospore 9 hrs. after sowing. × 334.

FIG. 2. *R. tenera*, 2 days old. × 334.

FIGS. 3–11. *R. tenera*, from 4 to 17 days old. × 334.

FIG. 12. *Lomentaria uncinata* Menegh., carpospore. × 334.

FIGS. 13–15. *L. uncinata*, 6 days old. × 334.

FIG. 16. *L. uncinata*, an unusually large specimen, 6 days old. × 213.

FIGS. 17–19. *Champia parvula* Harv., plantlet resulting from the germination of a carpospore, 8 days old. × 334.

FIG. 20. *C. parvula*, holdfast of a plantlet growing on Polysiphonia. × 213.

FIGS. 21, 22. *Lomentaria uncinata*, bases of mature plants.

FIG. 23. *Chondria tenuissima* (Good. et Wood) C. Ag., tetraspore shortly after it was sown. × 233.

FIGS. 24–28. *C. tenuissima*, plantlets developed from carpospores, from 2 to 4 days old. × 233.

FIG. 29. *C. tenuissima*, plantlet resulting from the germination of a tetraspore, 10 days old. × 233.

FIG. 30. *C. tenuissima* found growing on mature Chondria. × 233.

FIGS. 31, 32. *C. tenuissima*, 3 days old. × 233.

FIG. 33. *C. tenuissima*, 12 days old. × 213.

FIG. 34. *C. tenuissima*, 14 days old. × 213.

FIGS. 35, 36. *C. tenuissima*, 5 days old. × 213.

FIG. 37. *C. tenuissima*, portion of mature holdfast, stained with methyl [illegible] × 213.

FIGS. 38–40. *C. tenuissima*, holdfast of plants found growing on mature [illegible]dria, stained with methyl blue. × 333.

FIGS. 41–56. *Polysiphonia violacea* (Roth) Greville.

FIGS. 41–45. Carpospore and plantlets from 1 to 5 days old. × 400.

FIG. 46. Plantlet 7 days old. × 633.

FIGS. 47, 48. Plantlets found growing on *Scytosiphon lomentarius* Ag. [illegible]

FIG. 49. Plantlet, result of germinating carpospore. × 213.

FIGS. 50–56. Ends of primary rhizoids of plantlets 12 days old. × 400.

PLATE XXII.

FIGS. 1–10. *Polysiphonia violacea* (Roth) Grev.

FIG. 1. Plantlet growing on *Scytosiphoul omentarius* Ag. × 400.

FIG. 2. Plantlet 3 days old. × 400.

FIG. 3. Plantlet 9 days old. × 400.

FIGS. 4, 5. Plantlets 12 days old. × 233.

FIG. 6. Holdfast showing rhizoids springing from corticating cells. × [illegible]

FIG. 7. Plantlet 12 days old. × 213.

FIG. 8. Clasping disk of rhizoid of plant growing on *Zostera marina* L. × 400.

FIGS. 9, 10. Plantlets 9 and 11 days old. × 400.

FIGS. 11–29. *Spyridia filamentosa* (Wulf.) Harv.

FIG. 11. Carpospore. × 400.

FIGS. 12–18. Plantlets from 2 to 12 days old. × 400.

FIGS. 19–23. Bases of plantlets, showing primary cells and clasping disks. × 400.

FIG. 24. Base of rhizoidal branch (*c.* of *fig. 28*). × 213.

FIG. 25. Edge of mature holdfast, in optical section. × 157.

FIG. 26. Transverse section of frond, showing that the origin of rhizoidal branches is in the corticating cells; somewhat magnified.

FIG. 27. Procumbent branch, near *18a*, with rhizoidal outgrowths from central cell. × 333.

FIG. 28. Creeping branch, slightly magnified.

FIG. 29. Uncorticated secondary rhizoid with disk. × 333.

FIGS. 30–43. *Dasya elegans* (Martens) C. Ag.

FIGS. 30, 31. Germinating tetraspore. × 400.

FIG. 32. Segmenting carpospore 2 days old. × 400.

FIGS. 33–35. Segmenting tetraspore. × 400.

FIGS. 36–38. Plantlets 12 days old, resulting from the germination of tetraspores. × 400.

FIGS. 39–42. Plantlets 17 days old, resulting from the germination of carpospores; *figs. 39, 42* show only the root-cell and disk. × 400.

FIG. 43. Portion of mature holdfast, in optical section. × 233.

PLATE XXIII.

FIGS. 1–9. *Dasya elegans* (Martens) C. Ag. Plantlets resulting from the germination of carpospores; in some cases only the primary root-cell and disk are shown; from 12 to 20 days old. ×400.

FIGS. 10–23. *Ceramium rubrum* (Huds.) C. Ag.

FIG. 10. Germinating carpospore 2 days after sowing. × 633.

FIGS. 11–17. Plantlets from 3 to 8 days old; in *figs. 15, 16* only the basal cells and primary rhizoids are shown. × 400.

FIG. 18. Plantlet found growing on Polysiphonia. × 400.

FIGS. 19, 20. Plantlets 9 days old. × 400.

FIG. 21. Plantlet growing on Polysiphonia. × 400.

FIG. 22. Plantlet 5 days old, showing early branching of the primary rhizoid. × 400.

FIG. 23. Holdfast of a rather young plant. × 213.

FIGS. 24–28. *Ceramium strictum* (Harv).

FIG. 24. Carpospore 36 hours after it was sown. × 400.

FIG. 25. Plantlet 3 days old. × 233.

FIGS. 26, 27. Plantlets about 7 days old. × 400.

FIG. 28. Primary root-cell and disk of mature plant. × 400.

FIGS. 29–32. *Callithamnion Borreri* Ag., plantlets from 2 to 6 days old. × 213.

FIG. 33. *C. Borreri*, plantlet showing basal cell and rudimentary disk, 6 days old. × 213.

FIG. 34. *C. Borreri*, primary disk of plantlet 9 days old. × 333.

FIG. 35. *C. Baileyi* Harv., mature holdfast, in optical section. × 213.

FIGS. 36, 37. *C. Borreri*, base of plantlets 6 days old, each showing primary disk. × 400.

FIG. 38. *C. Borreri*, plantlet 6 days old. × 213.

FIG. 39. *C. Baileyi*, a portion of a mature holdfast. × 100.

FIG. 40. *Spermothamnion Turneri* Aresch., branch with clasping disk. × 213.

FIG. 41. *S. Turneri*, plantlet 6 days old; nine cells of the filament are not represented. × 213.

FIGS. 42, 43. *S. Turneri*, mature holdfast. × 213.

BRIEFER ARTICLES.

A PRACTICAL REFORM IN THE NOMENCLATURE OF CULTIVATED PLANTS.

Some years ago the Society of American Florists adopted Nicholson's *Dictionary of Gardening* as its authority for the names of cultivated plants until *Index Kewensis* should be completed. *Index Kewensis* has been finished for several years, but no florist, nurseryman, or seedsman has standardized the names in his catalogue until in the case about to be described. Moreover, no tradesman, so far as I know, has ever tried to be absolutely consistent in his names or to follow any one botanical authority. Nevertheless, the seedsmen, nurserymen, and florists are bringing up the perplexing problems of nomenclature, making resolutions, formulating rules, appointing committees, and adopting standards. An intelligent minority is always pressing for reform. Standards are adopted and no one follows them. Will they ever be followed? Some say no, and affirm that there are essential elements in trade that will always make horticulture and botany conflict more or less. I have long thought otherwise, and now have to record an experiment that seems to show an entirely practical way of standardizing the nomenclature of trade catalogues.

It seemed to me very important that some particular catalogue should be compared with *Index Kewensis*, and every name altered to conform with it. Every name not found in *Index Kewensis* should then be compared with Nicholson's *Dictionary of Gardening* and harmonized with that, if possible. This process has actually been followed in the catalogue of F. H. Horsford, of Charlotte, Vt. The case has proved to be an interesting one, and its main features should be put on record, for some of our best horticultural firms, I believe, are willing to standardize their catalogues, if only they are shown just how to do it. We should bear in mind that the principles of nomenclature, so familiar to every botanist, are entirely unfamiliar to the busy horticulturist with a living to make.

At the outset one might readily imagine that any nurseryman, seedsman, or florist who has access to *Index Kewensis* and Nicholcan do this kind of work himself for his own catalogue. This idea will have to be modified. Some tradesmen can do it themselves, but most cannot. However, all the important ones can hire it done, and cheaply. It is a job that would be exceedingly dry and uncongenial to many excellent business men. Competent students, however, can be found at the universities who are making their way, and would be glad of such work. A thousand names can be standardized for five dollars, at the rate of twenty cents an hour for twenty-five hours. This does not include the task of rearranging names in alphabetical order, or the reading of proof (as some cataloguers may prefer to do this themselves), but only the work of supplying the information necessary to the cataloguer.

There are about seven hundred species in the Horsford catalogue, and only twenty-five of those names are not to be found in *Index Kewensis* or Nicholson. This is less than 4 per cent., which is surprisingly low when one reflects on the great number of novelties since 1893: but Mr. Horsford sells largely of native plants, and these have received comparatively few trade names. Moreover, a goodly proportion of these twenty-five missing names are those of hardy native ferns. *Index Kewensis* has no ferns.

One barely begins to compare the names of a catalogue with *Index Kewensis* when he is confronted with an important problem of which this is a picture:

Alyssum saxatile Crantz = A. gemonense.
Alyssum saxatile Linnæus.

Now, how does the "standardizing clerk" know whether his employer has in his nursery the *Alyssum saxatile* of Crantz or of Linnæus? Probably he could give a shrewd guess. Possibly he may have both of the original descriptions at hand, and the plants also, but the chances are all against it. But, putting such considerations aside, this is a matter of identification, not of nomenclature, and the distinction between these two kinds of work must be grasped at the outset, or nothing can be done. The duty of the nomenclature clerk is clear. He assumes that the plant in the nursery is the one that *Index Kewensis* prints in ordinary type. The names in ordinary type, he knows, are considered by *Index Kewensis* to be the tenable ones, while all those in italics are synonyms. It is to Mr. Horsford's business interest to do all he can to find out whether the *Alyssum saxatile* in his nursery is properly named or is really *Alyssum gemonense*. Most tradesmen, however, do not have the time, the training, or the books to determine all their plants. Identification is the work of the botanist, and the day will come, I hope, when all the plants in the nurseries may be identified by specialists. Such work, however, is many times more costly than merely following a uniform system of nomenclature. The distinction between identification and nomenclature cannot be urged too strongly.

Another case is also interesting and occurs frequently:

Aconitum autumnale Lindley = A. Fischeri.
Aconitum autumnale Reichenbach = A. Napellus.

In this case *Index Kewensis* does not give any *Aconitum autumnale* in ordinary type, and therefore it recognizes no good species of that name. Here, again, the duty of the nomenclature clerk is clear, and he writes:

Aconitum autumnale (Lindl. or Reich.?).

It is not for him to decide whether the plant in the nursery is really *A. Fischeri* or *A. Napellus*. He has called the attention of the nurseryman to the question, and leaves it open. The nurseryman, perhaps, cannot settle the question while his catalogue is going to press, and he follows the suggestion of the nomenclature clerk literally. Perhaps he may not be able to settle the point for several years, but trade reasons are constantly urging him to get the point settled. Meanwhile it is consistent and honest to indicate a doubt. Two entirely different things have been cultivated under the name of *Aconitum autumnale*, but no one will suspect it if the fact were concealed. Honest doubt inspires confidence.

The next point will have great weight with the horticulturists. Mr. Horsford catalogues *Anemone montana* and *Anemone sylvestris* as two different things, but *Index Kewensis* says that the first is a synonym of the second. The nomenclature clerk allows Mr. Horsford to sell the two distinct things under the same names as before, but one of the entries now reads:

Anemone montana (*A. sylvestris* according to *Index Kewensis*, but horticulturally distinct with me).

This is perfectly clear, but too long, and a shorter way will be shown presently. The important things to note are two. First, the nurseryman is as free as before to differ in opinion from the official authority, but now he is consistent throughout, and supplies the opinion of the recognized authority as well as his own. Secondly, every name that appeared in the old catalogue appears in the new, but many of the old names now appear as synonyms or cross-references. No trade name that means money need be omitted. A shorter method of expressing a difference of opinion from official standards is to put an explanatory note at the beginning of the catalogue to this general effect: "Names in brackets show a difference of opinion." Thus we catalogue:

Anemone montana [*A. sylvestris*].

This means that *Index Kewensis* considers *A. sylvestris* to be the proper name of the species and *A. montana* the same thing, or perhaps only a botanical variety, while we consider that the two things are distinct for horticultural purposes. Instead of suppressing the opinions of others that conflict with our own, we tolerate them both, and place them side by side.

The commonest situation that needs change is shown by the following example: Mr. Horsford advertises for sale *Achillea Eupatorium*. *Index Kewensis* says this equals *A. filipendulina*. The best way for him to do is to advertise

Achillea filipendulina. (*A. Eupatorium.*)

This makes a great many changes in the alphabetical arrangement of species, and sometimes of genera.

The other way to do is to advertise under the old name, with the new name in parenthesis, and perhaps in different type, and an explanation in some prominent place of the device used consistently throughout the catalogue. This is a far less satisfactory method. The only thing that can be said for it is that it supplies the information. Doubtless it would be cheaper in some cases than revolutionizing a whole catalogue, but if a thing is worth doing at all it is worth doing well.

A few minor points may be briefly mentioned. The name of the author of a species would better not be given in trade catalogues unless absolutely necessary. It makes a catalogue look too dry and technical and unattractive. There is no officially accepted authority for popular names or for names of varieties, whether botanical or horticultural. *Index Kewensis* is not supposed to take account of anything below the species. Nicholson gives some varieties and also popular names.

It sometimes happens that *Index Kewensis* gives a species twice in ordinary type as if both were tenable. For instance, there is a *Campanula strigosa* of Vahl and a *Campanula strigosa* of Solander, both in ordinary type. In such a case the nomenclature clerk may write:

Campanula strigosa (Solander or Vahl?).

It is well to explain in the beginning of a catalogue which names are the proper ones and which are the synonyms. The latter are commonly in italics. It makes little difference how a catalogue is arranged, provided that there is a full index somewhere. Few indexes are full enough. The Horsford catalogue has no index, and there are seven departments, the arrangement being alphabetical under each department. The fact that there are seven departments should therefore be prominently stated, and the seven departments listed in the space of an inch or two in such a way that the mind can take in the whole scheme.—WILHELM MILLER, *Cornell University.*

THE BOTANICAL GARDEN AND INSTITUTE IN PADUA.

THE readers of the BOTANICAL GAZETTE may be interested to hear something of the ancient Botanical Garden of the University of Padua, instituted by the Venetian Senate in a decree of the twenty-ninth of June, 1545, through the wise forethought of Francis Bonafede in 1543.

The director, Professor P. A. Saccardo, who has recently improved the Institute and the Garden, published some interesting notices upon the 350th anniversary of its foundation from which I take the greatest part of this note.[1]

Professor Saccardo's activity turned, in the first place, to increase the library, initiated in 1770 by one of his predecessors, John Marsili, and enriched afterwards by Professor Bonato and Professor De Visiani, so that it contains already more than 10,000 volumes. Among the books, besides about forty periodical reviews and many valuable works, I must mention the oldest botanical book with instructive figures, viz., *Herbarium Apuleji Platonici*, printed in Rome in 1479.

The director has filled up during recent years the series of works on the floras, especially on the foreign ones, to make easier the labor

[1] SACCARDO, P. A.: L'Orto botanico di Padova nei 1895 (anno CCCL dalla sua fondazione). Padua. 1895. Quarto, with one topographical and eight heliotype plates.

of setting in order the herbaria, which he was then disposed to begin and which is now well advanced.

A hall, built in 1842 as a greenhouse, was arranged in 1892 to contain the general herbarium, consisting of 396 packets disposed horizontally in appropriate compartments of two great cases, with about 24,000 species represented by 60,000 specimens; the Dalmatian herbarium, composed of 37 packets with 2500 species and 10,000 specimens; and the cryptogamic herbarium, composed of commercial collections and those presented to the University.

The phanerogamic herbarium, especially from the Venetian provinces (65 packets, 3500 species, 10,000 specimens) is Saccardo's own and is placed in a great hall which was adapted in 1880 as a laboratory and contains also collections and materials necessary for scientific instruction. The students do their laboratory work there, under the attendant's guidance.

The mycologic herbarium, which is also the property of the director, deserves particular mention. It is in the director's room, in 66 cases (of $36 \times 23^{\text{cm}}$), and represents more than 30,000 specimens, many of which come from mycologists, some very rare. Saccardo's herbarium and mycologic library (300 volumes and 2300 pamphlets) are the important scientific material with which that clever mycologist wrote the classic *Sylloge Fungorum*. In the director's room near the library are the archives of the garden where there are the interesting autographs of Malpighi, Prospero Alpino, Cesalpino, and Pontedera.

Professor Saccardo has also increased the collection of portraits of botanists, a collection initiated by his predecessor, Professor Robert De Visiani. It is really well furnished, especially through gifts made by Baron Todaro, of Palermo, the son of the late botanist Augustin Todaro. It contains about 600 portraits, among which there are several of American botanists and of botanists who have studied the American flora.

In the lecture hall, built in 1842, which can contain two hundred men, there are portraits of seventeen professors of botany, in oil or black-and-white. Three weekly lessons on general botany are given in this hall by the titular professor; two free professors give free lessons (two a week) especially to naturalists and chemists; Dr. Adrian Fiori, attendant to the chair, delivers a course on cryptogams and plant pathology; and the writer delivers a course on plant physiology, with applications to agriculture.

So much for the Institute; but a great deal might be added as to the Garden and its greenhouses, which are rich in interesting plants[2] Classic plants are a *Chamærops humilis* L. var. *arborescens*, 9.5^{m} high, planted about 1585, and visited September 27, 1796, by Goethe, wherefore it is known as "Goethe's palm tree;" a *Tecoma grandiflora* Del., admired by Goethe for its beautiful flowering; a very old *Vitex Agnus-castus* L. (about 345 years old); an *Araucaria excelsa* R. Br. 20^{m} high, kept in a special greenhouse; many very beautiful trees (*Gymnocladus Canadensis* Lam., *Gingko biloba* L., *Diospyrus Lotus* L., *Carya olivæformis* Nutt., etc.). The greenhouses also are furnished with beautiful plants, among them an *Astrocaryon Chonta* Mart., a *Cycas circinalis* L., a *Cycas revoluta* Thunb., a *Pandanus utilis* Bory, a *Livistona australis* R. Br., many Cactaceæ and Orchideæ.

More than 5700 plants are cultivated in pots, to which we must add 110 old trees in the open air, 412 younger trees and shrubs, and 26 old greenhouse trees. — J. B. DeToni, *Padua, Italy.*

CONTRIBUTIONS FROM MY HERBARIUM.

Crataegus Sauratonae, n. sp. — A small tree $3-4^{m}$ in height, with an oval crown and ascending or spreading branches, the branches generally very crooked, as well as the slender twigs; twigs ash-gray in color, and armed, though sparingly, with stout gray or reddish spines, the twig of the season glabrous and red-brown: leaves glabrous, $2-5^{cm}$ long, obovate or elliptic, or rhombic-ovate, acute and sharply serrate above the middle, mostly entire towards the narrow base, with three or four pairs of prominent veins; the slender petiole $0.5-1^{cm}$ long; stipules, bud scales, and floral bracts not conspicuously enlarging, and early deciduous: flowers in rather small glabrous corymbs; sepals entire, lanceolate, glabrous; pedicels $1.5-3^{cm}$ long, glabrous, the red fruit about 12^{mm} in diameter, or more; styles four or five.

Related to *Crataegus collina* Chapm., and separated from it by having smaller glabrous foliage, sharply serrate leaves, and larger fruit. This species has been collected in wet flats along streams in the Sauraton mountains of North Carolina; on the tributaries of the Neuse river, in Granville county, N. C., and along streams in Caswell county, N. C.; growing with *Crataegus viridis* L., the white oak, and shag-bark hickory.

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[2] For the accounts of these see R. de Visiani: Di alcune piante storiche del giardino di Padova. Padua, 1856.— G. B. DeToni: Alberi e frutici ragguardevoli nei giardini di Padova. Padua, 1887.

CRATAEGUS COLLINA Chapman, which has been reported by Mr. C. D. Beadle from the mountains of North Carolina, is not uncommon in that state as far eastward as Durham county, generally growing along the edges of fields or in coppice woods.

CRATAEGUS VAILIAE Britton seems to be quite distinct from the closely related *C. uniflora* Moench., often having long, erect, virgate branches, and becoming a tall shrub; while *C. uniflora* is generally lower, seldom more than 1^m in height, with spreading or horizontal branches. It is found in North Carolina as far eastward as Durham and Raleigh.

Crataegus Chapmani (Beadle), n. comb. *Crataegus tomentosa Chapmani* Beadle, BOT. GAZ. **25**: 36. 1898. This tree is clearly worthy of specific rank. The much broader leaves and more prominent veins, smaller fruit in larger corymbs, and more slender spines separate it at once from all forms of *C. tomentosa*. In leaf characters and especially in the numerous pairs of prominent veins there is much resemblance to *C. punctata* Jacq. I find *Crataegus Chapmani* to be not uncommon in Ashe county, N. C., and Grayson county, southwestern Virginia.

FRAXINUS PROFUNDA Bush.—This tree has hitherto been reported only from the lower part of the Mississippi valley and the Gulf region. I have observed it, however, in a few places along the Atlantic coast as far north as Great Pungo swamp, Washington county, N. C. So far as noticed on the Atlantic coast the tree is confined to the largest river swamps, and the deeper flat swamps with stiff soils, growing with oaks, hickory, and occasionally loblolly pine.

QUERCUS TEXANA Buckl.—I reported this tree as occurring east of the Alleghany mountains, in this journal two years ago (**24**: 376.) in the Piedmont region of the Carolinas and Virginia. I have subsequently detected it on the Atlantic coastal plain, in Onslow county, N. C., within twenty miles of the Atlantic coast, where I saw a magnificent group of trees, some of the specimens being 35^m high, and more than 1^m in diameter.

HICORIA CAROLINAE-SEPTENTRIONALIS Ashe.—This tree proves to be not uncommon in certain portions of the Piedmont regions of the South Atlantic states. It prefers dry, rocky soils, steep declivities, and the crests of sandy ridges, though it occasionally enters lowlands. Along a narrow range of low slaty and rocky hills which extends with interruptions from Maryland to middle South Carolina the tree is frequent; while in central and northwestern Georgia it is more common, and it occurs, though locally, in middle Tennessee.

FOTHERGILLA MONTICOLA Ashe.—The recent discovery of this local shrub at Chapel Hill, N. C., makes another station for it about 150 miles east of any previously reported locality. It grows there on a rocky hillside with *Rhododendron Catawbiense* Mx., and the chestnut oak. This is also the most eastward station for *Rhododendron Catawbiense* Mx. Dr. J. K. Small reported Crowder's mountain as being the most eastward station, but Chapel Hill is 140 miles further east, and has an elevation 1000 feet less than that of Crowder's mountain, being only 500 feet above sea level. The *Systematic Flora* (**2** : 42) gives the plant as occurring only at high elevations. This rhododendron is also found abundantly along the Oconneechee hills, twelve miles northwest of Chapel Hill, and at a slightly higher elevation. With it at this place is *Aconitum reclinatum* Gray, one of the most local species of the genus, and hitherto supposed to be confined to higher elevations, 5000–6500 feet, in the southern Alleghanies.—W. W. ASHE, *Biltmore, N. C.*

TWO NEW MICHIGAN FUNGI.

Tubaria luteoalba, n. sp.—Pileus 1–2.5^{cm} broad, thin, convex, becoming plane, finally centrally depressed, the margin sometimes becoming partly or wholly upturned, hygrophanous, white, creamy or yellowish, silky-squamulose near the margin from the remains of the veil, margin striate when moist: stem 1.5–2^{cm} long, 0.3–0.5^{cm} thick, hollow, slightly enlarged at base, whitish, silky, downy at base, often curved: lamellæ adnato-decurrent, 0.2–0.4^{cm} broad, subdistant, at first nearly white but soon ochraceous from the spores: spores elliptical, 4–5 × 6–8μ.—On decaying stems and leaves of weeds and grass, on low wet ground near Michigan Agricultural College, April 1897.

This fungus resembles *T. furfuracea* in form and habit, but is smaller, lighter in color, and in every way more delicate than that species. The spores are also smaller and lighter in color. From *T. autochthona* it differs in its larger size, form of stem, and habitat. The veil sometime forms a fibrous zone on the stem. It has not been collected in any other locality.

Galera crispa, n. sp.—Pileus 1.5–3.5^{cm} broad, membranaceous, persistently conico-campanulate, subacute, uneven and somewhat rivulose.

ochraceous-brown on disk, lighter toward the margin which becomes crenulate and upturned in older specimens, slightly pruinose at first, rugulose and a little paler when dry: lamellæ slightly adnexed, not crowded, rather narrow, interspaced with anastomosing veins, much crisped, at first nearly white, then becoming ferruginous from the spores: stem 7–10^{cm} long, 0.1–0.3^{cm} thick, tapering from the somewhat bulbous base, yellowish-white, pruinose at base, hollow, fragile: spores 10μ broad, 12–16μ long.— In grass in dooryards and pastures, June and July.

The specific name is based on the peculiar character of the gills which are always crisped as soon as the pileus is expanded. Professor Charles H. Peck, to whom specimens of the above fungi were referred, and who very kindly reported on them, suggested that this might be a variety of *G. lateritia*, unless the peculiar character of the lamellæ proved to be constant. The fact that specimens possessing this character have been collected in the same localities during the past three seasons seems sufficient proof of the constancy of this character, and consequently, would indicate that this form is worthy of specific rank.
—B. O. LONGYEAR, *Michigan Agricultural College.*

MEXICAN FUNGI. II.[1]

THE following species of Ustilagineæ were collected by me in 1896 and 1898, and all, except the three marked with an asterisk, have been examined by P. Hennings, of Berlin.

USTILAGO RABENHORSTIANA P. Henn. On *Panicum filiforme.* Guadalajara, Oct. 12, 1896.

USTILAGO PAMPARUM Speg. On *Setaria.* City of Mexico, Oct. 2, 1896.

USTILAGO ULEI P. Henn. On *Chloris submutica.* City of Mexico, Oct. 2, 1896.

USTILAGO DIETELIANA P. Henn. On *Tripsacum dactyloides.* City of Mexico, Oct 1, 1896.

USTILAGO HILARIÆ P. Henn. On *Hilaria cenchroides.* City of Mexico, Oct. 2, 1896.

USTILAGO ÆGOPOGINIS P. Henn. On *Ægopogon cenchroides* P. Henn. City of Mexico, Oct. 3, 1896.

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[1] For previous paper see BOT. GAZ. **24**: 23. 1897.

USTILAGO BRUNKII Ell. and Gallw. In culms of *Andropogon perforatus*. Cardenas, Mexico, Oct. 22, 1898.

USTILAGO NUDA (Jens.) Kell. & Swingle. On *Hordeum vulgare*. Toluca, Sept. 19, 1898.

USTILAGO CENCHRI Lagerh. On *Cenchrus* (*tribuloides?*). Cardenas, Oct. 22, 1898.

Ustilago Holwayana P. Henn., n. sp. Soris in spicis, eas destruentibus, longe cylindraceis, primo membrana pallide cinerea velatis, atris; sporis subglobosis vel ellipsoideis, subacutangulis, atro-violaceis, 13–16 × 12–15 μ; episporo verrucoso, atro.—In spicis *Paspali velutini*. Patzcuaro, Michoacan, Oct. 19, 1898.

Ustilago Andropoginis-hirtefolii P. Henn., n. sp. Soris in paniculis, ovaria destruentibus, longe cylindraceis, membrana cinnamonea vestitis dein pulverulentis, atro-olivaceis vel fusco-olivaceis, 9–14 μ; episporio granuloso verrucoso.— In paniculis *Andropogonis hirtifolii pubiflori*. Patzcuaro, Michoacan, Oct. 20, 1898. Apparently quite distinct from all the species known on Andropogon.

Ustilago Panici-proliferi P. Henn., n. sp. Soris in paniculis, eas omnino destruentibus, membrano cinerea tectis, dein pulverulentis atro-olivaceis; sporis subglobosis, acutangulis, pallide olivaceo-fuscis, intus granulatis, 7–9 μ; episporo levi.— In paniculis *Panici proliferi acuminati*. City of Mexico, Oct. 10, 1898. This species differs from *U. Panici-miliacei* (Pers.) Wint. in its much smaller spores, and in having the sori covered with an ashy-gray membrane; and from *U.* [illegible]*losa* P. Henn. and *U. pustulata* Tr. & Earle in its smooth lighter colored spores. The sori and general appearance are also different.

*USTILAGO PARLATOREI F. de Wald. On *Rumex Mexicanus* Meissn. Toluca, Mexico, Sept. 19, 1898.

*USTILAGO TRITICI (Pers.) Jens. On *Triticum vulgare*. City of Mexico, Oct. 11, 1898.

*USTILAGO ZEÆ (Beckm.) Ung. On *Zea Mays*. City of Mexico, Oct 10, 1898.

CINTRACTIA AXICOLA Cornu. On *Fimbristylis*. Cuernavaca, Mexico, Sept. 26, 1898.

CINTRACTIA LEUCODERMA (Berk.) P. Henn. On *Rhynchospora*. Jalapa, Mexico, Oct. 3, 1898.—E. W. D. HOLWAY, *Decorah, Iowa*.

OPEN LETTERS.

TO BRYOLOGISTS.

THE undersigned desires to make a full canvass of all working bryologists and collectors of mosses, both American and foreign, believing that a better general acquaintance will further both the friendly and scientific spirit. To this end the following data are cordially solicited from all who have not already sent them:

1. Name in full; age; vocation.
2. Average time your vocation annually permits you to devote to mosses.
3. A list of your bryological publications; also a list of exsiccati you may have distributed.
4. Have you worked on any foreign moss flora? State in which geographical region you are most interested.
5. Which genera, or larger groups, are you making your specialty?
6. Are you desirous to have referred to you for critical examination species that fall in the line of your special interest?
7. Do you exchange mosses? If so, what material do you offer?

A circular embodying these questions was sent out in June of the present year. It was answered so approvingly and enthusiastically that I feel justified in making an additional effort to make the data as complete as possible, to put them when collected into permanent form, and to place them finally in the hands of each one interested.—JOHN M. HOLZINGER, *Winona, Minn.*

CURRENT LITERATURE.

BOOK REVIEWS.

Botanical teaching.

A TOPIC which is or should be of deep interest to professional botanists in our higher institutions is the nature and quality of the botanical instruction in the secondary schools. Many of them have shown their interest by personal endeavor to help teachers to improve the scope of their courses and to secure proper equipment of laboratories for instruction. This endeavor is bearing fruit, and the rapid improvement in botanical teaching augurs well for future development. Besides the innumerable fugitive addresses to teachers' associations and institutes, several modern text-books and laboratory guides are helping along the good work.

Professor Ganong now contributes a book which will do much to assist teachers to strengthen their botanical work. This book, *The Teaching Botanist*, has a pedagogical purpose, as distinct from the texts and laboratory handbooks. It consists of two independent parts. The first is made up of eight essays on botanical pedagogics, full of excellent ideas, useful suggestions, and earnest admonitions. Many of these reforms botanists have long been advocating. Now that the ideas are pithily put and in permanent form they will become more widely efficient than heretofore. Those who have proclaimed the new gospel will be delighted to have this book to which they may refer teachers seeking such help. It will save reams of letters and hours of talk.

If we may choose among the good things in this part, the essays on "What botany is of most worth," "On things essential to good botanical teaching," and "On some common errors prejudicial to good botanical teaching" are probably the most useful. But the suggestions on drawing and description, on laboratories and their equipment, on collections, and on books are excellent and sure to be helpful.

The second part consists of "an outline for a synthetic elementary course in the science of botany," conforming to the principles elucidated in the first part. This course consists of two divisions, the first to elucidate general principles, and the second to present the chief features of the larger groups of plants. The course is divided into various topics with a series of questions and directions which might be put directly into the hands of pupils. These are followed by notes regarding the materials required and remarks on the pedagogical import of the various points called for.

Doubtless few teachers will want to follow exactly this course, nor does the author expect them to do so; but many will certainly derive great help in selecting from it the topics appropriate to their own conditions and having clearly before them the didactic value of the laboratory work.

It may be worth while to point out that the author's principles, which one cannot escape, do not compel the conclusion that it is best to begin the elementary course with a study of seeds. The teacher who now begins by introducing the student to the simple algæ need not feel that he must abandon this method. The excellent principles presented in the second essay may be as well developed by another method. And it is only fair to say that Professor Ganong advises each teacher to make out his own course.

University men will do well to read Dr. Ganong's essays and recommend the book to every teacher of botany.—C. R. B.

Buds and stipules.

Sir John Lubbock has published a book with the above title in the well-known International Scientific Series.[1] There is little or no attempt to give anything new, but rather to place before the world in a somewhat popular form the most interesting results of his previous study.[2]

The author was led to study stipules by the observation of Vaucher that some rock-roses have stipules and others not; the question arose: why? The study of stipules led on to a study of buds, especially their protective structures.

The order of the chapters does not seem particularly logical, and there appears to be more repetition than is needed, even in a popular work. The first two chapters deal in a general way with buds and stipules; the third with the development of leaves and stipules, *i. e.*, their organogeny. The fourth chapter takes up the protection of buds, which may be by older leaves, leaf bases or petioles, stipules, hairs, resins. Detailed examples are given under each head. The author thinks that the shape of leaves is often determined by the shape of the bud or seed, and he attempts to explain in this way why some leaves are lobed and others not. For example, oak buds are short, the leaves are folded in the bud, and hence are lobed. There is possibly a confusion here between *post hoc* and *propter hoc*.

There is a long chapter on the structure of buds, many species being mentioned. Chapter six treats of the forms of stipules, and it is shown how great a variety there is. In the seventh chapter the author discusses the subsidiary uses of stipules. Their general use he conceives to be to cover and protect the buds. They are often important also as organs of photosynthesis;

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[1] Lubbock, John: On buds and stipules. Crown 8vo. pp. xix + 239. *pl. 4. figs.* 340. London: Kegan Paul, Trench, Trübner & Co. L't'd. 1899. 5s.

[2] On stipules. Parts I–IV. Jour. Linn. Soc. Bot. **28, 30, 33**. 1890, 1894, 1897.

they may become tendrils, or spines, or glandular organs; or they may be rudiments, looking back to organs of use in another form.

There is a chapter also on the nature of stipules. There are three views as to what stipules are: (1) they are appendages of the leaves (Van Tieghem, Baillon, Gray); (2) they are autonomous organs, analogous to leaves (Lindley); (3) they are an integral part of the leaf. Lubbock holds the third view. The first view he regards as untenable because stipules originate independently of leaves and often before them; the second because the stipular bundles are derived from the foliar bundles.

The book is full of illustrations and very suggestive, though it seems that there is too great a certainty as to just what everything is for.—HENRY C. COWLES.

An ecological text-book.

AMONG THE recent text-books for secondary schools none is so dominated by the new ecological standpoint as the book just issued by Dr. John M. Coulter. This is one of the series of "Twentieth Century Text-books," in course of publication by Messrs. D. Appleton & Co.[3]

It is the first of a pair of books, each representing work for half a year, but independent. The second, with the title *Plant Structures*, is to be issued shortly. It is to be dominated by morphology as the first is dominated by ecology. In the judgment of Dr. Coulter the order in which he issues the books is the proper one for presentation in an elementary course. This sequence is likely to meet with the criticism that the student, in ignorance of plant structure and without wide acquaintance with plant groups, is unable to appreciate ecological phenomena and principles. The author believes the advantages which counterbalance the disadvantages are (1) the obtaining of a true conception of plants in nature, (2) acquaintance with the large problems of plant physiognomy, and (3) the avoidance of the use of the compound microscope at the outset.

Though the physiognomy of vegetation is an interesting and perhaps most important phase of botany, it is doubtful whether at the present time the subject is well enough organized to justify its dominating an elementary course. It is still more doubtful whether it will be possible for many years to find teachers capable of presenting it. Granting the ecological aspect to be the ideal botanical course, the question is whether we are yet far enough away from the floristic or pseudo-taxonomic teaching to justify an attempt to reach so remote an ideal. The writer has already committed himself to the view that the simpler morphology and physiological topics should be first presented in an elementary course and therefore only states the pedagogical problem. To its solution Dr. W. F. Ganong contributes interesting arguments in a book elsewhere reviewed.

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[3] COULTER, J. M: Plant Relations, a first book of botany. 12mo. pp. x + [illegible]. *figs. 206.* New York: D. Appleton & Company, 1899.

Readers of *Plant Relations* will be impressed by the terse and lucid style. Though the utmost condensation has been necessary, the author has preserved a simplicity of language and has attained a degree of accuracy which leaves nothing to be desired. The book is also striking in the number and beauty of its many illustrations, of which a large part are original. Among the finest ones are those derived from Schimper's recent treatise, *Pflanzengeographie.*

A very useful pamphlet of twenty pages, embodying suggestions to teachers for the use of the book, is designed to accompany it. It contains helpful remarks regarding the laboratory and field work which the author intends, of course, shall be prosecuted as the foundation for the study of the text.

We are sure that live school-teachers will welcome this book because it presents a new view of the plant world, valuable for instructional purposes and hitherto too much overlooked. University teachers will receive it gladly because it emphasizes one of the vital aspects of botany, and makes more effective the crusade against the cut and dried formalism of "analysis." — C. R. B.

Cytological technique.

INVESTIGATIONS upon the structure of protoplasm demand not only extreme skill in mechanical manipulation but also a knowledge of the principles underlying fixing, staining, and other details of microtechnique. A recent book by Dr. Alfred Fischer puts the whole subject of microtechnique upon a firmer and more philosophical basis and gives an up-to-date discussion of modern theories of protoplasmic structure.[4]

Part I, on fixing agents, considers in detail the solutions in common use and describes their action upon the various cell contents, as peptone, proalbumose, nucleic acid, nuclein, etc., etc. The numerous experiments with substances of known chemical composition should be of value in determining what are to be regarded as artifacts and what as structural elements of the tissue. The closing chapter on the fixation of cell contents will be found especially helpful by those engaged in cytological work.

Part II (128 pages) is devoted to staining. Both theory and practice are considered in detail. Some of the topics are as follows: The washing out of the fixing agent, and its significance in theories of staining; staining with simple staining solutions without differentiation; double staining with simple solutions; simultaneous double staining with mixed stains; impregnation; objections to the physical theory of staining; chromatin and the fundamental doctrines of staining. Here again experiments upon substances of known chemical composition occupy a large part of the space.

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[4] FISCHER, ALFRED.: Fixirung, Färbung und Bau des Protoplasmas. Kritische Untersuchungen über Technik und Theorie in der neueren Zellforschung. 8vo. pp x + 362. *pl. 1. figs. 21.* Leipzig: Gustav Fischer. 1899. *M* 11.

Part III deals with the structure of protoplasm. Spindles, centrosomes and radiations are thoroughly discussed and artificial figures are compared with those occurring normally. Chromatin is treated in the paragraphs on granules. The various theories of the structure of protoplasm, as the granule theory, the network theory, the filar theory, and the foam-structure theory, are critically reviewed.—CHAS. J. CHAMBERLAIN.

Knuth's Handbook.

STUDENTS of the interrelations between plants and their pollinators, constituting a branch of what the Germans call "biology," and what Americans are coming to call "ecology," have learned their first lessons in large part from Christian Konrad Sprengel, once rector of the Lutheran Stadtschule at Spandau, Charles Darwin, and Hermann Müller, late Professor in the Realschule at Lippstadt. Some years since, Sprengel's book, "Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der Blumen," was reissued by Professor Paul Knuth, of the Ober-Realschule at Kiel; and an English translation of Müller's "Befruchtung der Blumen" has brought his work within reach of many persons not familiar with the German language. It appears that the original edition is no longer procurable, and Professor Knuth set himself the task of revising and reissuing it. The progress of this branch of science has been so great in the last quarter century, however, that he has found it better to write an independent work,[5] based on Müller's writings, but brought up to date.

For reasons not perfectly clear to the uninitiated, this has been divided into three parts: an introduction and bibliography, pollination observations in Europe and the arctic region, and extra-European studies in the ecology of flowers. The first two volumes are now issued, in three parts, ending with a comprehensive index. The third volume is announced as in course of preparation, and will be received with no little satisfaction on its completion. Few lines of botanical work are so fascinating or so accessible to the beginner as pollination studies, and with this book before him he should be able readily to sift the known from the new in his observations, so that the latter may be added to the former in suitable published form. It is doubtless too much to hope for an English translation, but the absence of one is only one more indication of the urgent need of a working knowledge of German on the part of every student ambitious to distinguish himself in modern scientific work.—WILLIAM TRELEASE.

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[5] KNUTH, PAUL: Handbuch der Blutenbiologie unter Zugrundelegung von Hermann Müller's Werk, "Die Befruchtung der Blumen durch Insekten." Engelmann. Leipzig. 1898–9.

MINOR NOTICES.

A MONOGRAPH of the hypogaeous fungi of California has been published by Mr. H. W. Harkness.[6] One hundred and eight species are described, of which fifty-eight are new, with five new genera. There is unfortunately no artificial key or synopsis, so that the work will be of little value except to the specialist, and we fear he will have occasion to complain of the brevity of the diagnoses of new genera and species. These rarely exceed two or three lines, with few or no explanatory remarks.—C. R. B.

F. V. COVILLE and G. N. ROSE have published a list of plants collected by Mr. and Mrs. Leonhard Stejneger on the Commander islands during 1895 and 1897. These islands bear no trees, due, the authors think, to the violent cold winds that sweep over them during the winter. The geographic relationship of the flora is primarily Kamchatkan, with strong Aleutian and arctic elements, and there is almost no insular specialization. The list is reprinted from *The fur seals and fur-seal islands of the North Pacific Ocean*, part 4, pp. 352–361. 1899.—J. M. C.

"BOTANIZING" is the title of the new book by the author of *The Botanical Collector's Handbook*, Professor W. W. Bailey, of Brown University.[7] It embraces the material of the former volume but is completely rewritten and greatly improved. The amateur will here find the completest and best directions for all kinds of botanical collecting. The author has been assisted by various specialists, some making only general suggestions, others preparing the sections relating to particular families of phanerogams and the other groups of cryptogams. Fifteen illustrations show useful apparatus for preservation of specimens. An index should have been added. The author (*in litt.*) asks readers to delete the words "the steppes of Asia or" on page 4, line 7.—C. R. B.

NOTES FOR STUDENTS

IN A PRELIMINARY PAPER read before the Botanical Society of America, at Columbus, Dr. Charles E. Bessey discussed the probable significance of apetaly and its connection with diclinism and diœciousness. He suggested that well-established plants, as shrubs and trees, as well as those which mass their flowers may dispense with petals. Petaly is apparently correlated with entomophily, and apetaly with anemophily. Apetaly and diclinism appear to result from progressively increasing aphanisis. Lists of apetalous and diclinous plants were given and discussed.

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[6] Proc. Calif. Acad. Sci. III. Bot. 1:241–286, *dbl. col. pl. 42–45*. 1899.

[7] BAILEY, W. W.: Botanizing: a guide to field-collecting and herbarium work. 8vo. pp. xiv+142. *figs. 15*. Providence: Preston & Rounds. 1899.

AT THE COLUMBUS MEETING of the A. A. A. S., H. L. Bolley presented in Section G a paper by Lawrence Waldron on "The occurrence of calcium oxalate and lignin during the differentiation of the buds of *Prunus Americana*." It was found that the crystals of calcium oxalate occur in quite surprising abundance in the meristematic tissues of the bud, and in the very youngest stages of the scales of the bud; and that the oxalate becomes lessened in proportionate quantity as the tissues develop. Lignification of the hairs and scales of the bud commences at a very early period of their development. While it is usually assumed that calcium oxalate is a waste product of metabolism, its occurrence in such large quantities in the meristematic cells of the bud and scales would seem to indicate a question as to whether it has not a definite value at this point at this particular time in the life history of the plant.

PROTHALLIA OF LYCOPODIUM are so rare that the present paper, although based upon only half a dozen specimens, is a valuable contribution.[8] These prothallia resemble those of *Botrychium Virginianum* as described by Jeffreys. A vertical section shows a limiting layer of colorless cells, above which are several cells invested by an endophytic fungus. The cells of the upper half of the prothallium are entirely free from the fungus. The antheridium is developed from a single superficial cell and at maturity does not project above the surface. The archegonium, also developed from a single superficial cell, projects considerably. There are six or eight canal cells, the lowest presumably a ventral canal cell.

The value of the prothallium as a taxonomic character is discussed at some length, and the writer concludes that species of Lycopodium which possess similar prothallia cannot, on that ground alone, be regarded as closely related. The prothallium of *Botrychium Virginianum* in form, structure, endophytic fungus, position of sexual organs and subterranean saprophytic habit resembles the prothallia of *Lycopodium*, but the resemblance merely shows how an appearance of genetic relationship may result from modifications due to a similar subterranean saprophytic habit.—CHAS. J. CHAMBERLAIN.

AN EXCEEDINGLY interesting account of the fertilization of *Batrachospermum Bohneri* by W. Schmidle[9] has recently appeared. This author has worked on material gathered in Germany and his conclusions, while agreeing with my own studies in respect to the presence and behavior of the chromatin res in the trichogyne and antherozoids, are very different as regards the activities of the nuclei and processes of fertilization.

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[8] LANG, W. H.: The prothallus of *Lycopodium clavatum* L. Ann. Bot. 13:279–317. 1899.

[9] W. SCHMIDLE: Einiges über die Befruchtung, Keimung, und Haarinsertion von Batrachospermum. Bot. Zeit. 57^1:125. 1899.

The antherozoids of *B. Bohneri* differ from any that I have ever seen in having almost invariably two nuclei. When the antherozoid fuses with the trichogyne the nucleus nearest to the point of application passes into that structure and is usually followed by the second, which however may remain behind in the antherozoid.

The trichogyne and carpogonium of *B. Bohneri* have only one nucleus which is in the carpogonium. One of the nuclei from an antherozoid passes the length of the trichogyne into the carpogonium through the constriction between the two structures. Fertilization takes places in the carpogonium, where the male nucleus fuses with the female.

The passage of the male nucleus causes the protoplasm in the trichogyne to gather into a peculiar dense mass which indicates a certain stage in the process of fertilization.

After fertilization the carpogonium becomes cut off from the trichogyne, and the latter may then contain one or more nuclei introduced from one or several antherozoids that fuse with it. Extensive fragmentation of these nuclei may occur later.

The phenomena described are in all respects compatible with our knowledge of the processes of fertilization. They accord fully with the accounts of Oltmanns and Wille for other members of the Rhodophyceæ.

I have realized for a long time that my account of Batrachospermum[10] left the problems considered in a very unsatisfactory state. The conditions that I described were exceptional and at variance with those so generally present in related groups of plants. However, at the time, I was convinced of the correctness of my position, and since that publication I have several times re-examined my material, always finding the same structures that I figured and described.

My chain of evidence is nevertheless incomplete in that I have not seen the mitotic figure which should give rise to the nucleus that I have supposed to be normally present in the trichogyne. Such a stage would be exceedingly difficult to find and recognize, and I have searched persistently for it. It is very important and even necessary for my views that its existence should be established. I have not been able to do this as yet, and in this respect acknowledge a weakness in my former position.

I do not wish to criticise the work of Schmidle further than to suggest the necessity of the most thorough studies in cytology in investigations of this sort. I wish we might have the details of nuclear fusion in fertilization and nuclear division in the antherozoid, together with an absolutely complete series of stages illustrating the process of fertilization.

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[10] Davis: The fertilization of Batrachospermum. Ann. of Bot. 10:49. 1896.

These can only be obtained from material very carefully killed and fixed, perhaps after special methods. I must believe that we shall not feel sure of the processes in Batrachospermum until the technique of the investigation is developed to a point much superior to that of either Schmidle or myself.—BRADLEY MOORE DAVIS.

BARTHOLD HANSTEEN publishes the extended results of his researches on the synthesis of proteids in green phanerogams in Pringsheim's *Jahrbucher*.[11] He used *Lemna minor*, *Vicia Faba*, and *Ricinus communis* as experimental plants. His summary we translate:

1. In general, at least, light plays no direct rôle in the synthesis of proteids in the bodies of green phanerogamous plants. In these the formation of proteids occurs in active cells without the influence of light and independent of the time of year, if only suitable conditions for growth be present.

a. Glutamin, asparagin, urea, ammonium chlorid or ammonium sulfate combine with available grape sugar or—at least the four last named nitrogen compounds—with the direct reducing sugar formed in the digestion of starch.

b. Urea or glycocoll generally unite either with available cane sugar or probably indirectly reducing sugar.

2. The chemical nature of the immediately available carbohydrate is not unimportant for proteid synthesis; on it primarily depends whether the formation of proteid is effected or not.

3. The various amides (amido-acids) or nitrogen compounds are generally not physiologically equivalent for proteid formation. The best suited for this purpose is urea, whose transformation into proteid occurs as energetically with cane sugar as with grape sugar. On the contrary, leucin, alanin, and creatin cannot be looked upon as materials so suitable for proteid making; for even under the most favorable conditions, and equally whether direct or indirect reducing sugar in suitable amounts is simultaneously accumulated in the cells, proteid formation from these compounds fails.—C. R. B.

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[11] Jahrb. f. wiss. Bot. 33: 417–486. 1899.

NEWS.

Mr. Abel A. Hunter has been appointed botanical collector for the University of Nebraska.

The University of Geneva has conferred the degree of Ph.D. *honoris causa* upon M. Casimir de Candolle.

Dr. Carl E. Correns has been advanced to the assistant professorship of botany in the University of Tübingen.

Dr. Wilhelm Figdor has been appointed docent for anatomy and physiology of plants in the University of Vienna.

Houghton, Mifflin & Co.'s fall announcements include a book entitled *Animal and plant lore*, by Mrs. Fanny D. Bergen.

Dr. J. M. Janse, of the botanical garden at Buitenzorg, Java, has been appointed professor of botany in the University of Leiden.

Dr. K. Giesenhagen, of Munich, has received from the government a subvention of *M*6000 for an investigating tour to Malacca.

Dr. L. Hiltner has been appointed director of the bacteriological laboratory of the biological division of the Imperial Bureau of Hygiene in Berlin.

Professor George W. Martin, teacher of biology in the Indianapolis High School, has been appointed professor of biology in Vanderbilt University, Nashville, Tennessee.

Mr. C. G. Pringle has returned from his fifteenth year in the collection of Mexican plants. His health has not been good, but, as usual, he returns with a collection of choice plants.

Dr. Paul Knuth, of Kiel, has returned to his home from his journey around the world. He has brought with him abundant ecological material from Java, Japan, and California.

In a recent fire which destroyed the entire stock of Jacob North & Co., in Lincoln Neb., all the unsold copies of Pound and Clements' *Phytogeography of Nebraska* were burned.

Dr. August N. Berlese, heretofore professor of botany in the University of Camerino, has been appointed professor of natural sciences in the [illegible]a Lyceum. Dr. J. B. DeToni has been appointed to the position thus vacated by Professor Berlese. Dr. DeToni's address, however, will still be Padua.

ON MAY 1 the north wing of the great temperate house at Kew Gardens was opened to the public. The center of this house was built in 1862, and the south wing in 1894. The building is 628 feet long, with a maximum width of 164 feet and a height of 60 feet. It covers one and two thirds acres, and has cost £43,000.

THE INTERNATIONAL scientific medal of the *Academie Internationale de Geographie Botanique* has been conferred upon Dr. N. M. Glatfelter, of St. Louis, for his work upon Salix, and upon Dr. Roscoe Pound, of Lincoln, Neb. for his phytogeographical researches. Fifteen investigators in Europe have been similarly honored.

THE LINNEAN MEDAL has been awarded to Mr. J. G. Baker, late keeper of the herbarium and library of the Kew Gardens, "for his services to botany during a long series of years, especially his writings on ferns and petaloid monocotyledons, serviceable alike to botanists and cultivators." The presentation occurred at the anniversary meeting, May 24.

THE MACMILLAN COMPANY announce for publication this autumn a *Handbook of North American Myxomycetes* by Professor Thos. H. Macbride, of the University of Iowa. The work includes descriptions of all species hitherto described from North America, with brief synonymy, accompanied by diagnostic notes. The work is to be illustrated by nineteen full-page plates.

BY THE cooperation of a local mountain club, Dr. R. von Wettstein, the director of the Vienna botanical garden, has been enabled to establish a biological experiment station in the Tyrolese central Alps near the "Bremer-Hütte" in the Gschnitzthal, at an elevation of 2300^{m}. A room in the cottage has been fitted up for a laboratory. Research will be directed first to the production of species by direct adaptation.

MR. C. L. POLLARD of the National Museum, aided by Professor E. L. Greene, has undertaken the distribution of authentic sets of North American Violaceæ. Forty sets are in preparation and the museum offers them in return for an equivalent amount of selected duplicates, either in the Violaceæ or other groups. Decades will be issued at irregular intervals, as rapidly as material can be secured and labels printed.

THE POSSIBILITY of obtaining separates of articles from periodicals often renders possible the carrying on of investigations apart from the great libraries. We welcome, therefore, every establishment which facilitates the diffusion of such literature. Mr. A. I. Eriksson, Tufts College, Mass., is going into the business of dealing in natural history books and author's separates

He will act as agent for authors who wish to dispose of any of their publications. A catalogue will be issued shortly.

AT THE MEETING of the St. Louis Academy of Science, held on the evening of October 16, Dr. H. von Schrenk presented some notes on *Arceuthobium pusillum*, which was found in Maine during the past summer, growing on the white spruce along the seacoast. The trees which are attacked form large witches' brooms, the branches of which are much longer than the normal branches. The manner in which the seeds are distributed was briefly described, and seeds were exhibited adhering to branches of the white spruce.

MR. O. F. COOK, of the Division of Botany, Department of Agriculture, has been detailed to make a preliminary examination of the plant products of Puerto Rico with reference to the introduction of new and useful tropical plants into that island. Mr. Cook is accompanied by Mr. G. N. Collins of the Department of Agriculture as photographer, and by Mr. George P. Gall, who is sent by the Smithsonian Institution to collect material for the National Herbarium. The expedition left New York on October 28 by the United States transport MacPherson.—*Science*, Nov. 3, 1899.

THE DISTINGUISHED French horticulturist Henri Lévêque de Vilmorin was stricken by apoplexy and died at Verrières on August 24, in his fifty-seventh year. He visited this country in 1893, when many of our botanists had the pleasure of meeting him at Madison, Wisconsin. Henry Vilmorin was not only the head of the historic house of Vilmorin & Co., the largest seed firm in France, but personally directed in large part the numerous experiments for the improvement of cultivated plants which the firm were continually conducting at Antibes, Verrières, and Ferme de St. Fiacre. Vilmorin was widely honored by scientific societies and has made valuable contributions to horticultural literature.

ON MARCH 3, 1899, a bill was passed by Congress providing "that on or before January 1, 1903, the fence around the Botanical Garden shall be removed; provided that at the first session of the LVI Congress the Joint Committee on Library is directed to report a bill embodying a plan for removing the Botanical Garden to another location." The present Botanical Garden is far too restricted to serve the purpose of a national establishment. With the rapid development of such gardens as those at New York and St. Louis, the national garden at Washington becomes insignificant. The proposition to remove it is most commendable. The chief danger arises from the fact that a site suggested is so far removed from the centers of botanical work as to prevent the new garden from becoming of the greatest scientific service.

MR. WALTER T. SWINGLE, of the U. S. Department of Agriculture, has returned from eighteen months' travel in Europe, North Africa, and Asia Minor, where he has been studying the agriculture and horticulture, with a view of introducing new agricultural industries into America. The journey was under the direction of the Section of Plant and Seed Introduction of the Division of Botany. He paid particular attention to the date industry of the Sahara desert, and to fig culture as practiced in Greece and Asiatic Turkey, since both of these industries are likely to be introduced very shortly into our own southwestern states. Incidentally he noted many points of great botanical interest. The caprification of the fig is still practiced as described by Aristotle more than two thousand years ago, and a careful study of the commensalism and symbiosis of the fig plant and Blastophaga is by no means superfluous, but on the contrary very much needed, since all previous students have studied it at the same time of the year, and many doubtful points remain to be cleared up. Mr. Swingle is going to California very shortly to study the fig industry of our own Pacific coast.

Professor W. A. Setchell, Dr. W. L. Jepson, Mr. L. E. Hunt, and Mr. A. A. Lawson, of the University of California, have returned to Berkeley from a botanical expedition to Unalaska. Dr. Jepson studied the flowering plants, Professor Setchell and Mr. Lawson the flowerless plants, while Mr. Hunt, who is in the Civil Engineering Department, determined altitudes and took the photographs of plant communities, etc. The party remained at Unalaska for eight weeks and carried out its work as planned, collecting thoroughly in the neighborhood of Unalaska bay, making extensive field notes and securing a fairly full collection of photographs. Professor Setchell left Unalaska for about three weeks, on a trip to St. Michael and Cape Nome, collecting plants of all kinds and making notes as to points of distribution and ecology. Returning, the party went from Unalaska to Sitka along the coast, collecting at Unga, Karluk, Kodiak, Orca, Juneau, and Sitka. They were thus able to trace many plants of the shores along a considerable portion of the Alaska coast, and to note the changes in habit and the difference in altitudinal distribution. There is a very considerable amount of material accumulated and it will not be known until it is carefully worked over how much of it is new, or just to what extent it will throw light on matters of distribution. The collections of marine algæ, taken in connection with other collections made in Alaska, Washington, California, and Mexico during the last four or five years, it is hoped, will indicate the limits of the various algal floras of the Pacific coast of North America when they are properly determined and tabulated, and will afford the basis for some exact inquiry into the causes of demarcation.—*Science*, Oct. 13, 1899.

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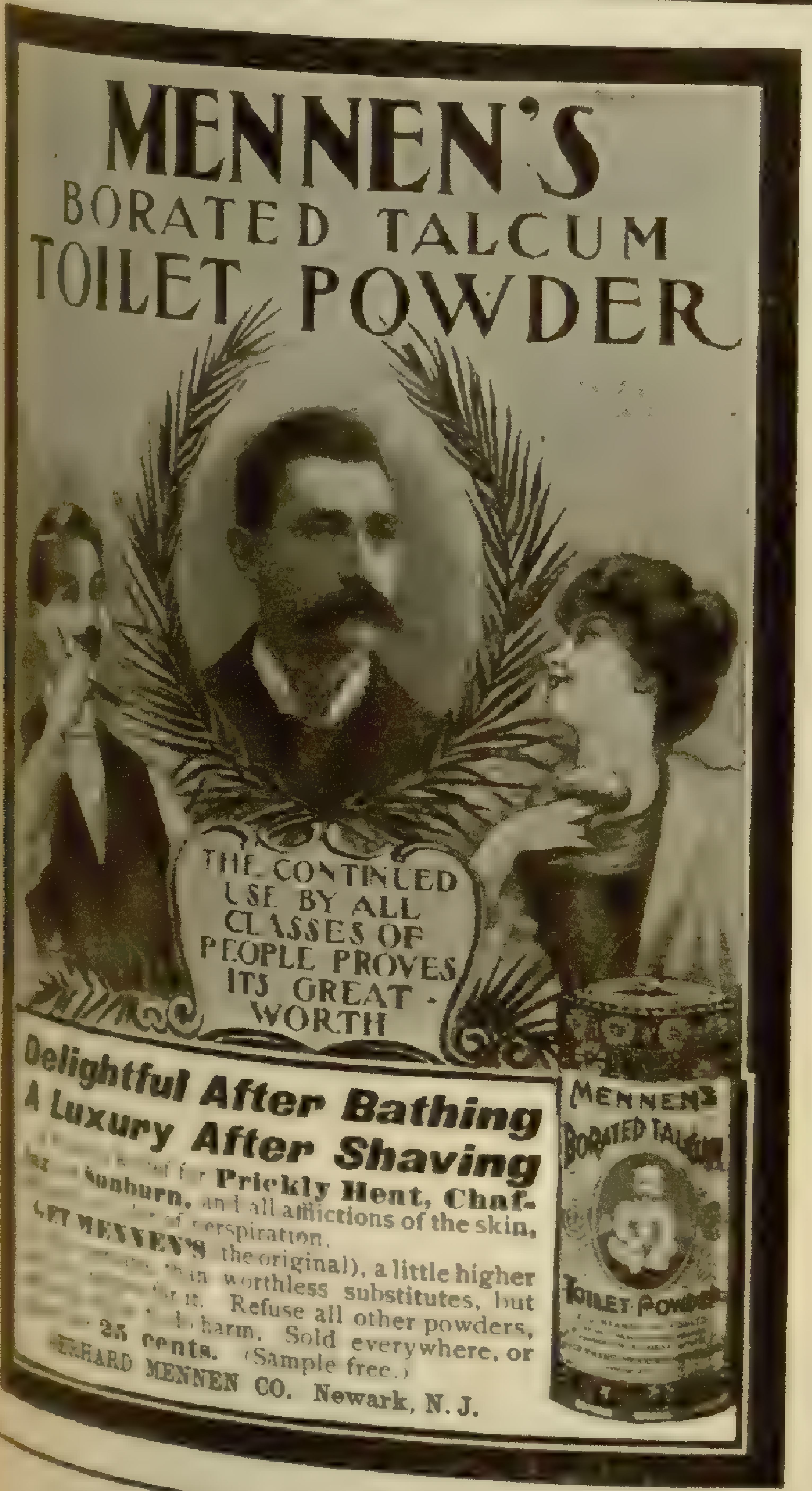

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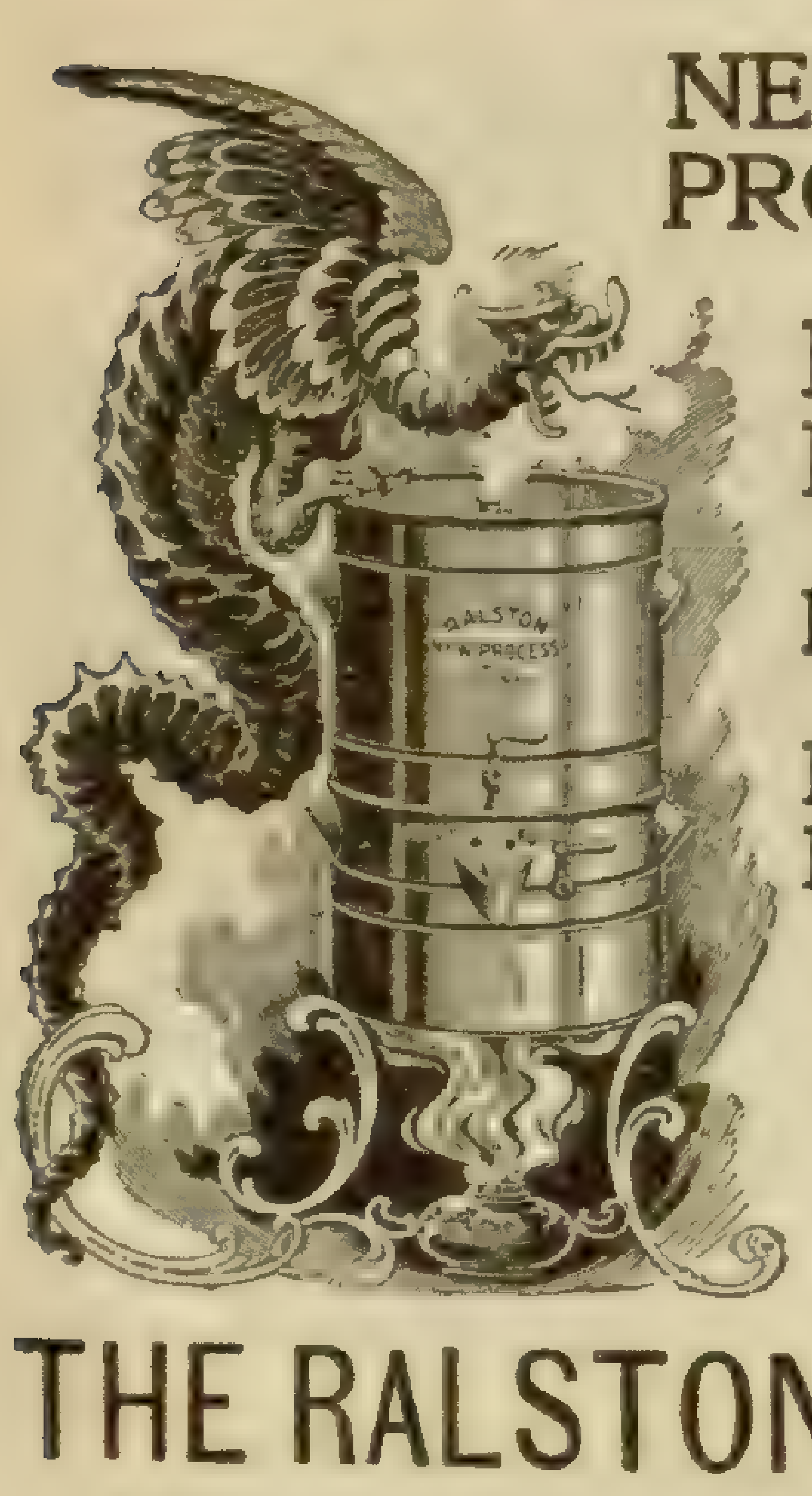

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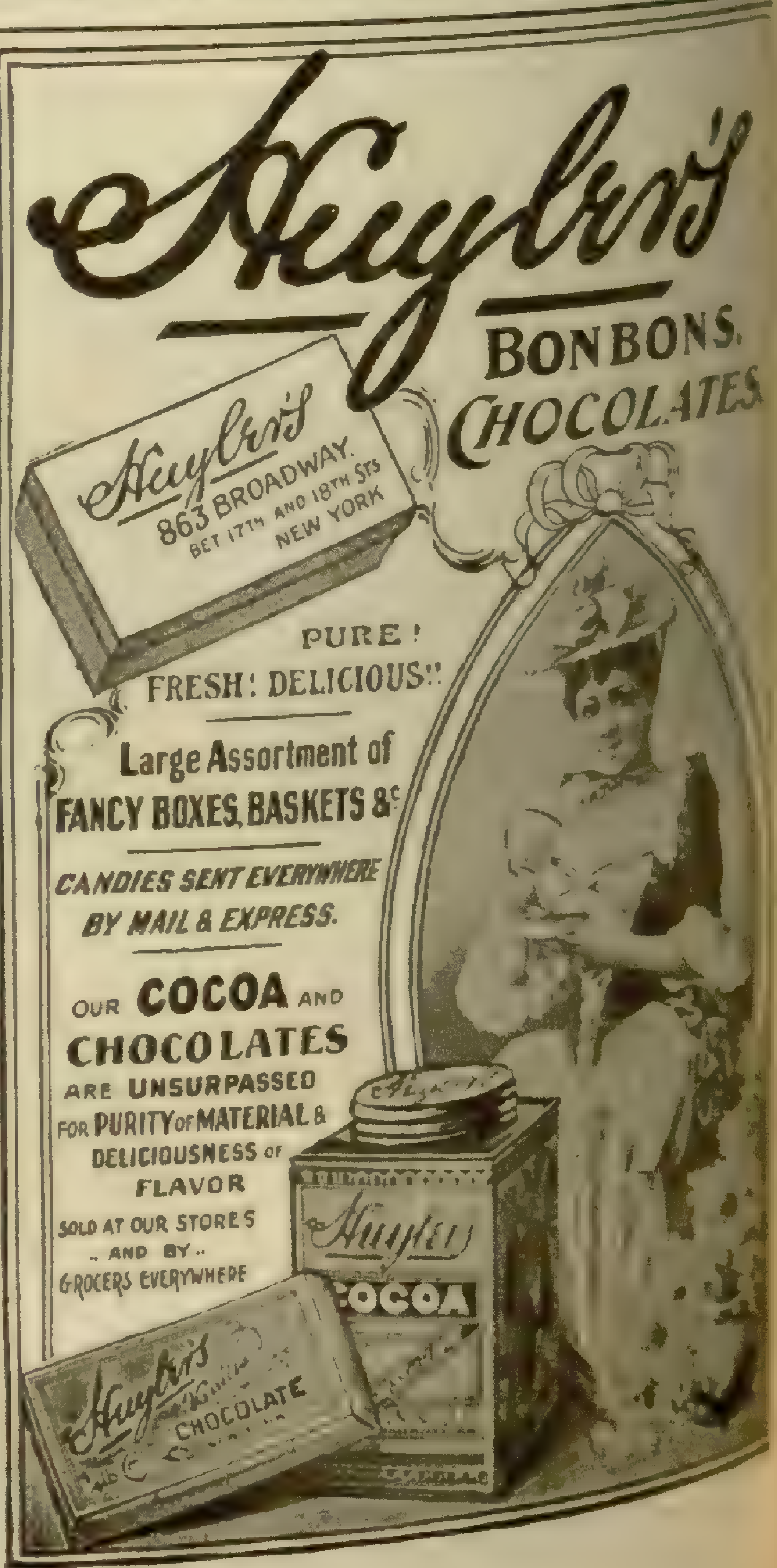

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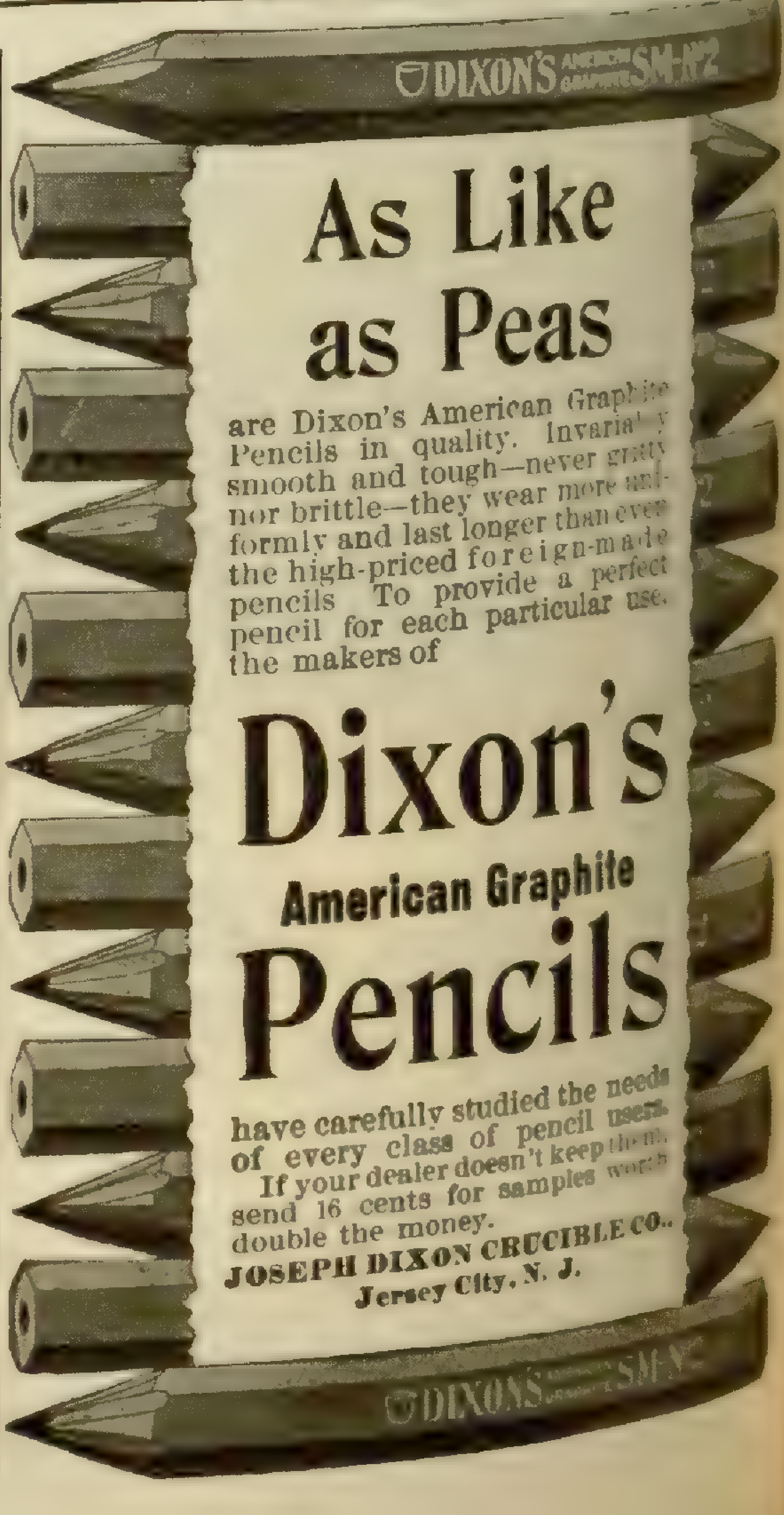

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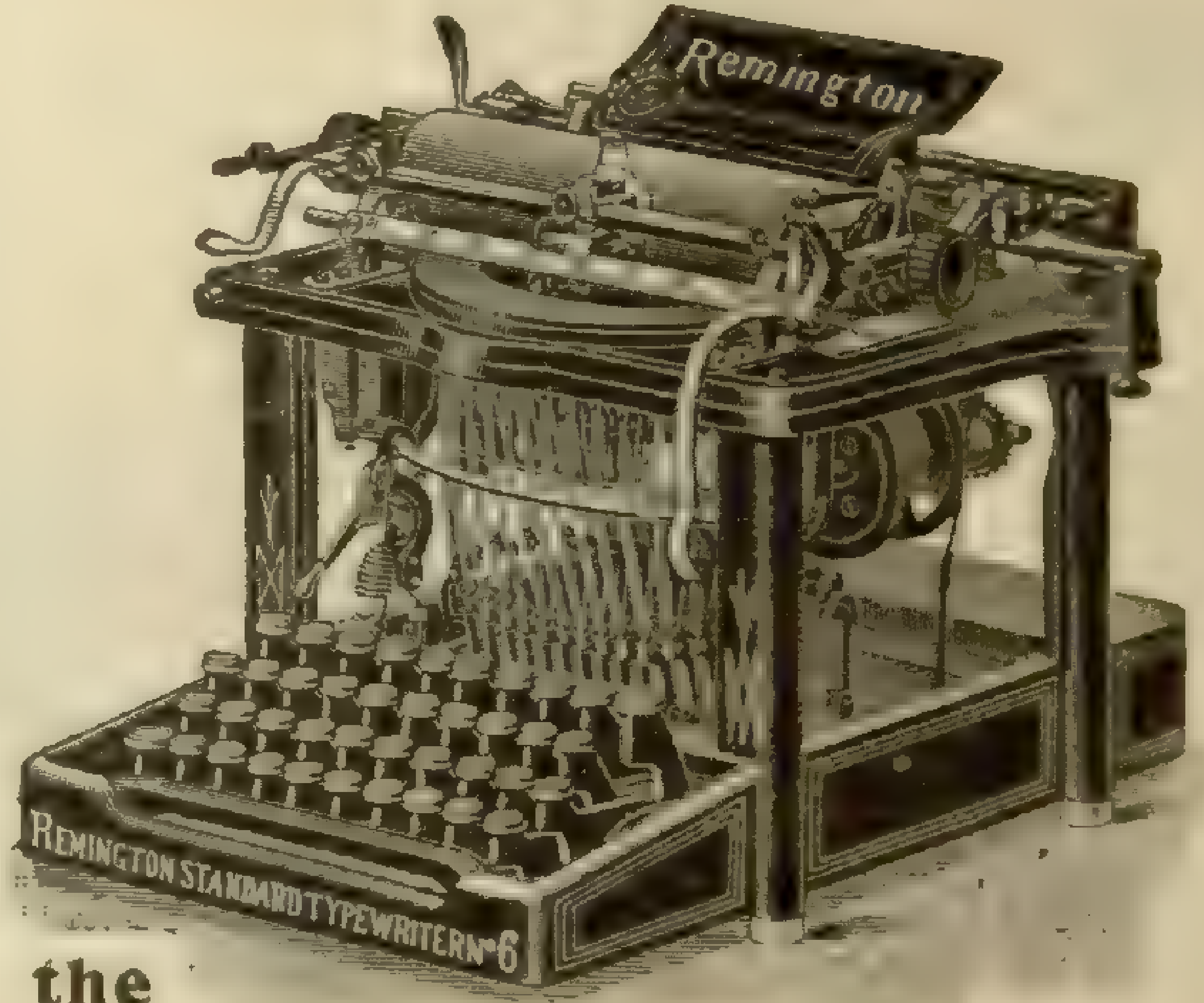

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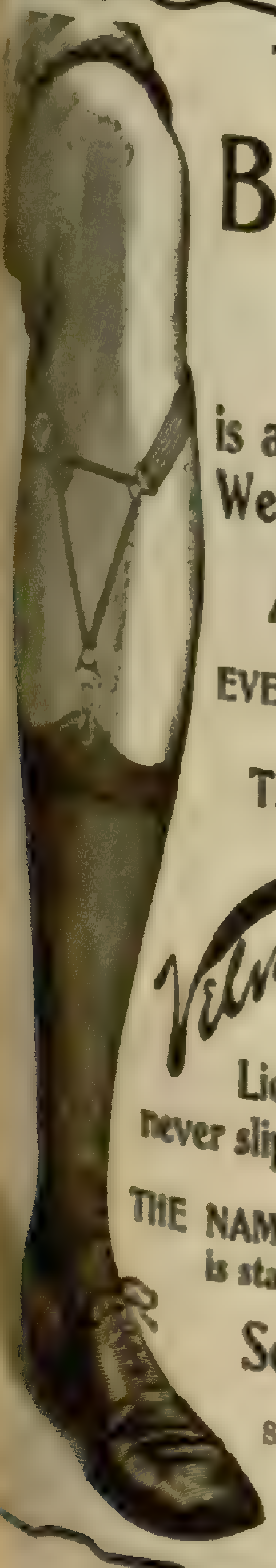

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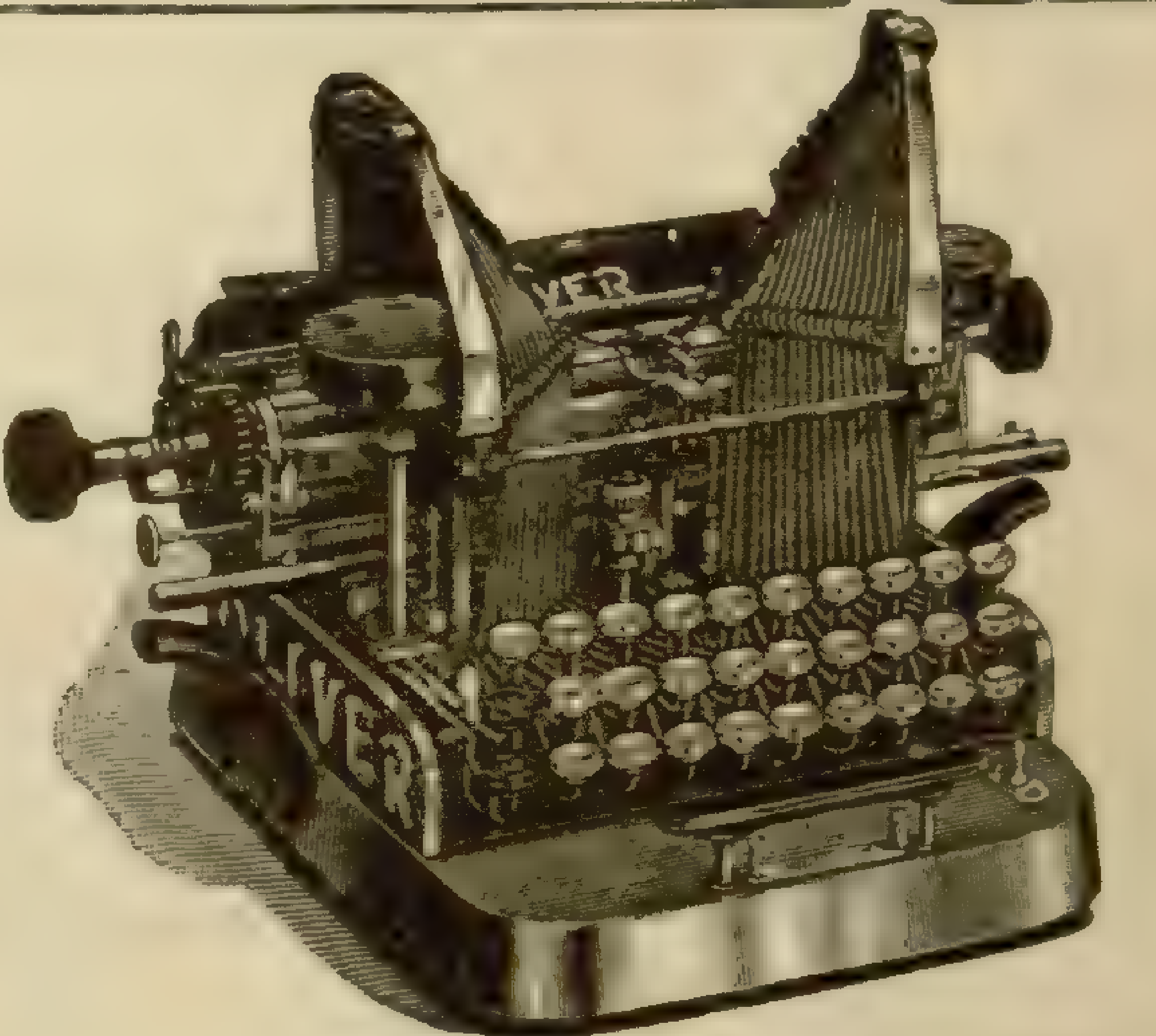

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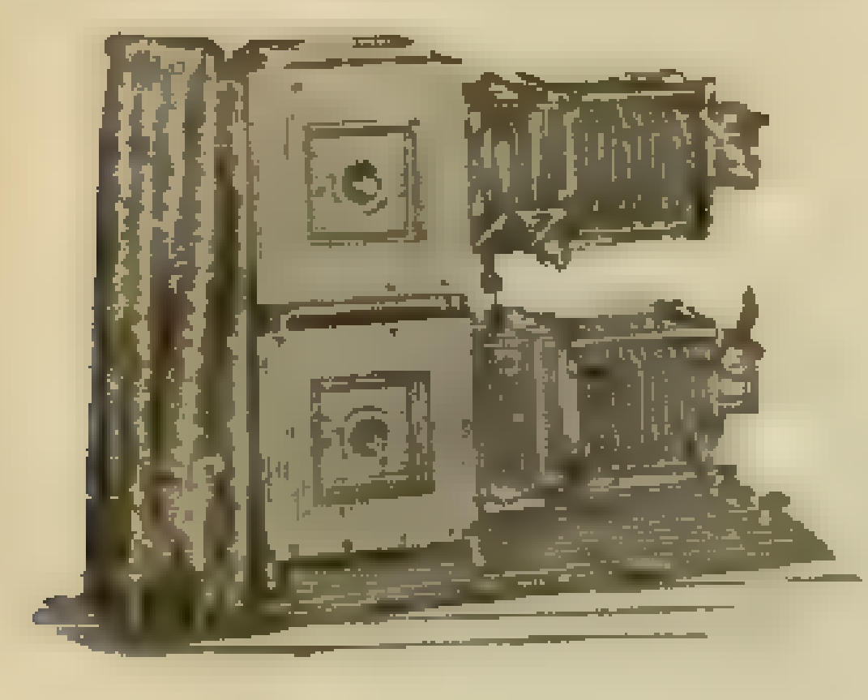

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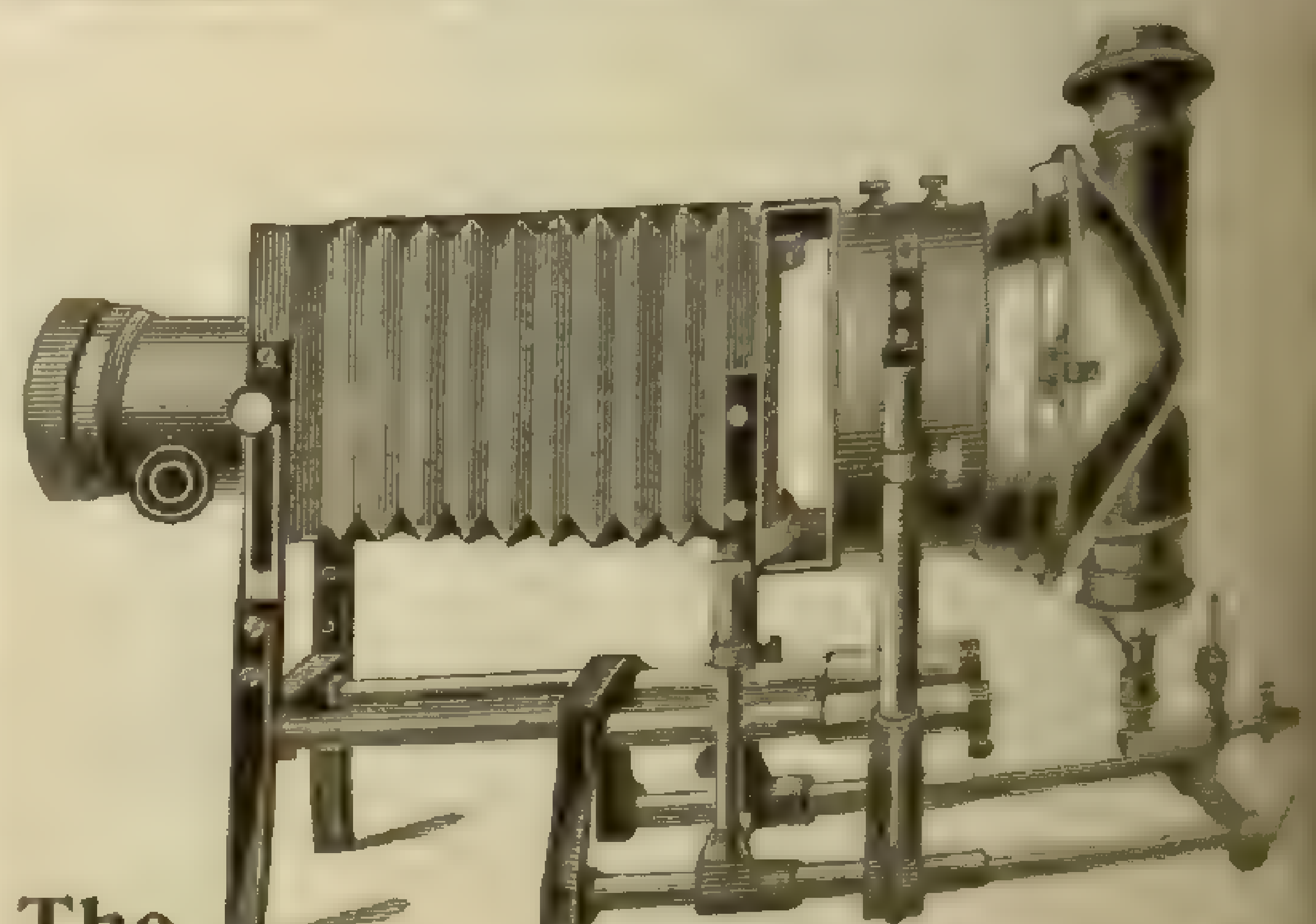

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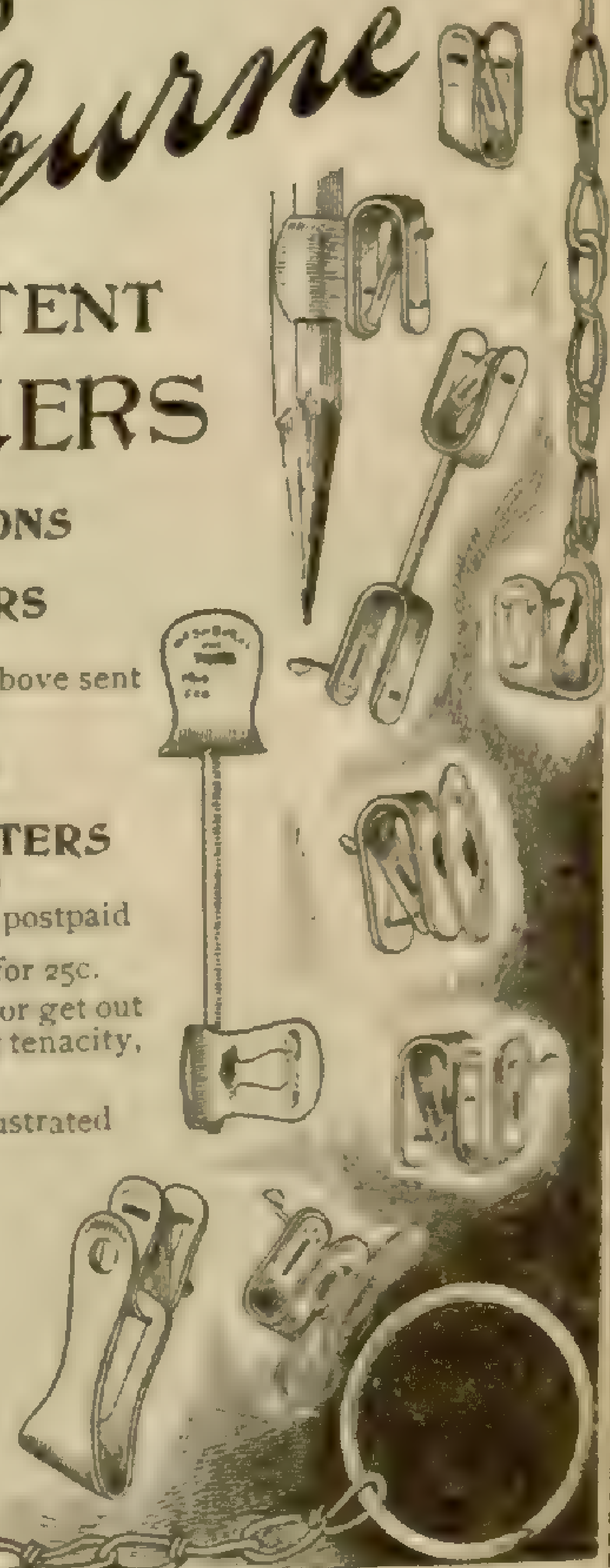

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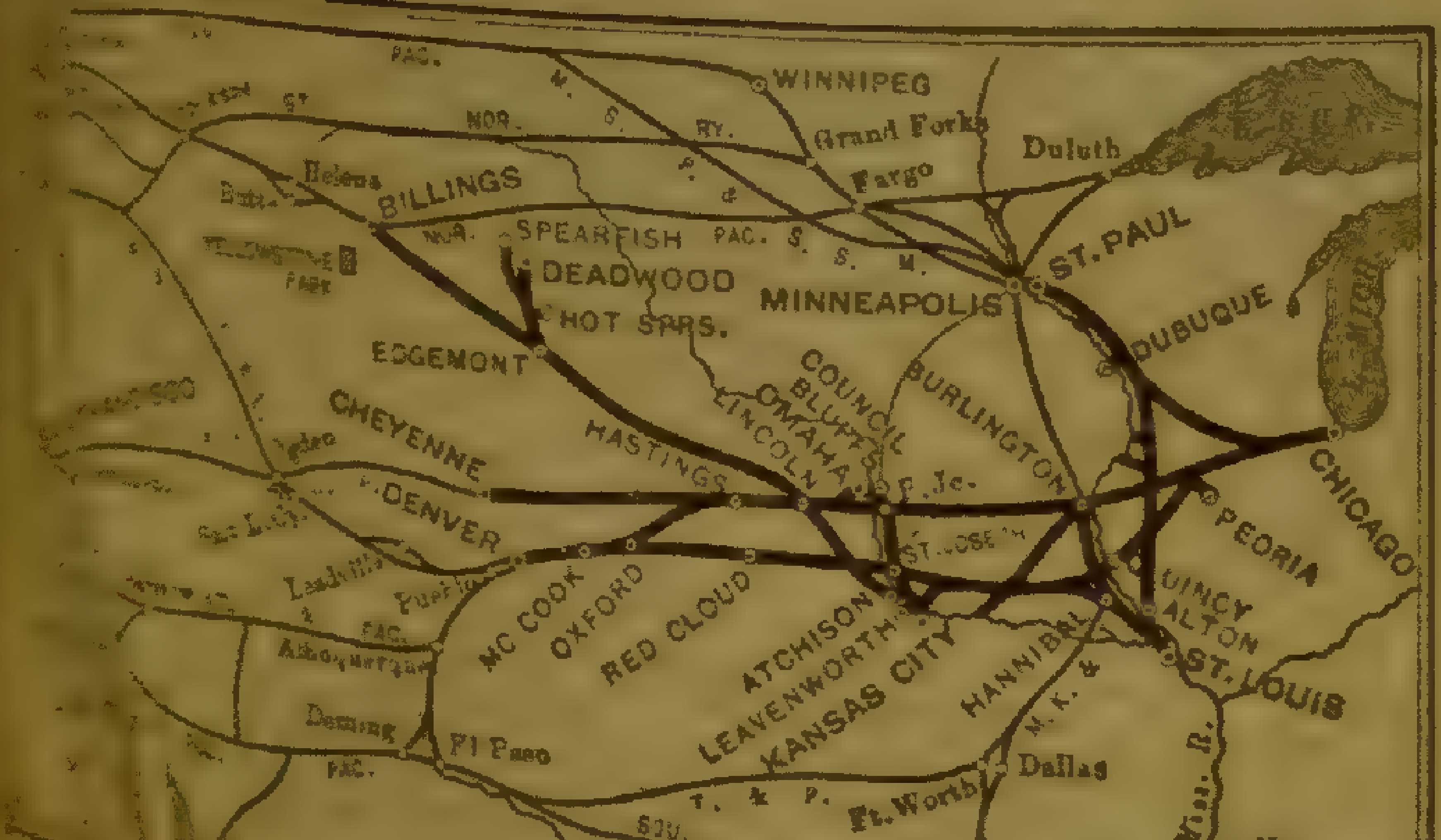
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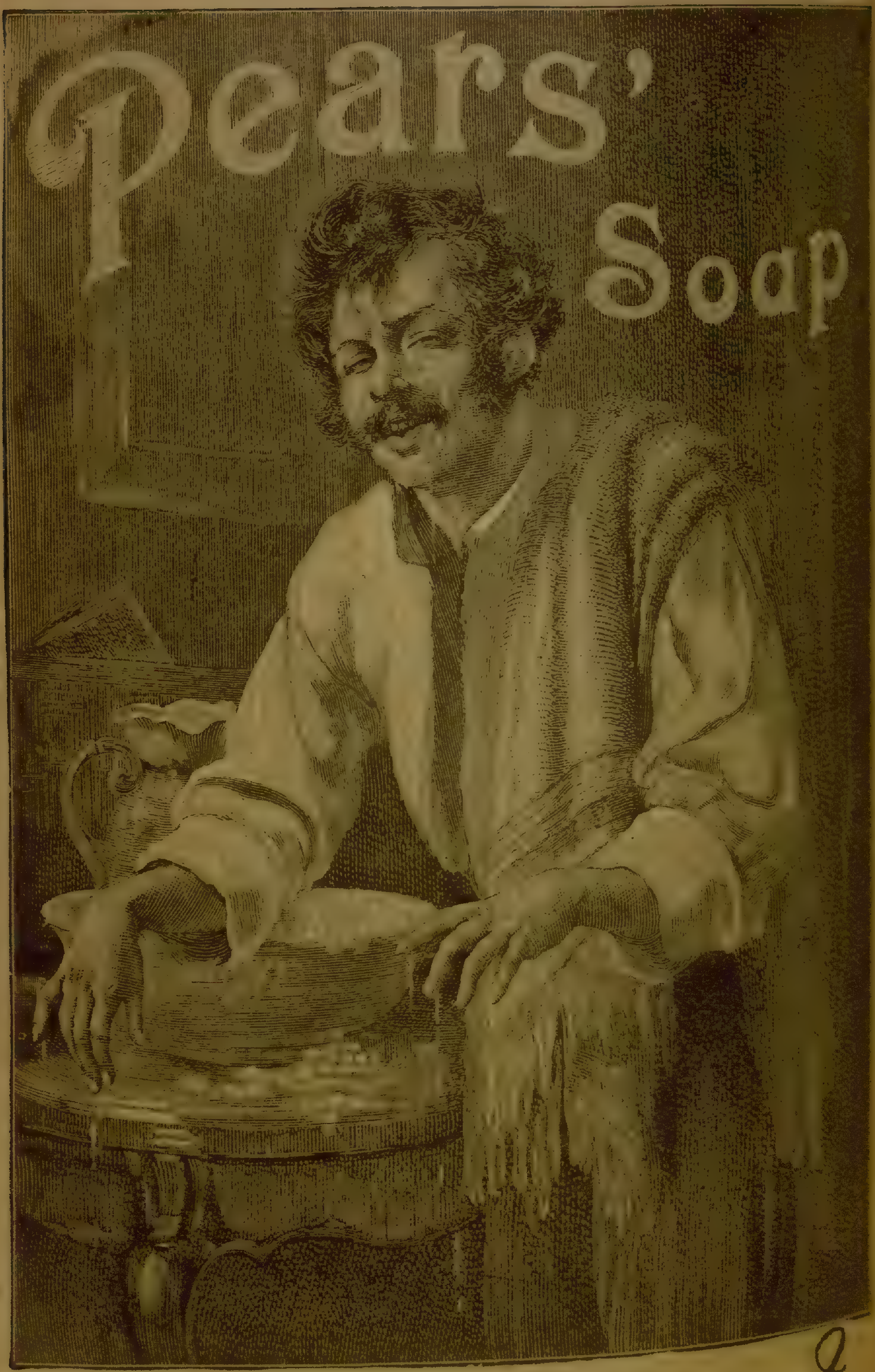
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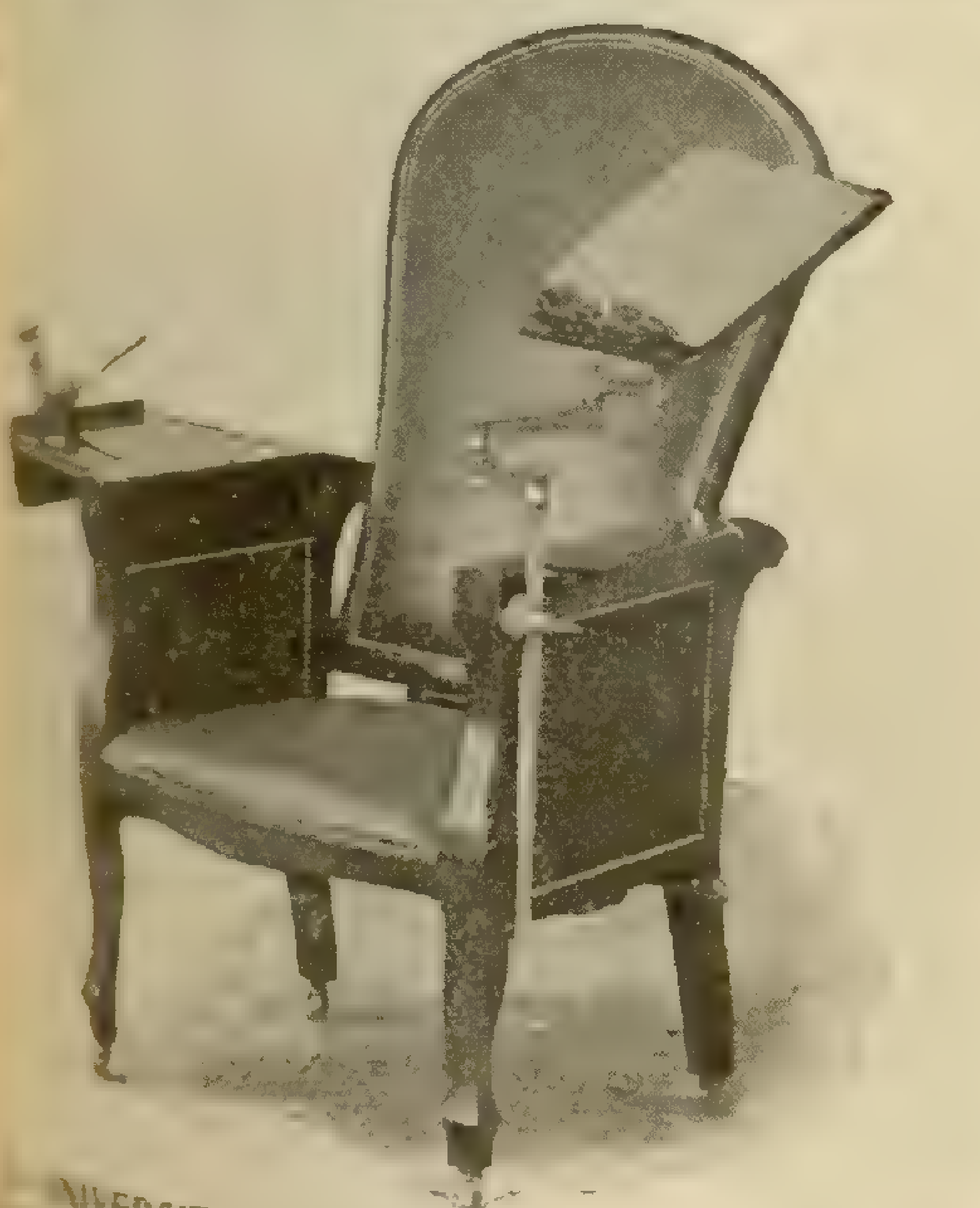

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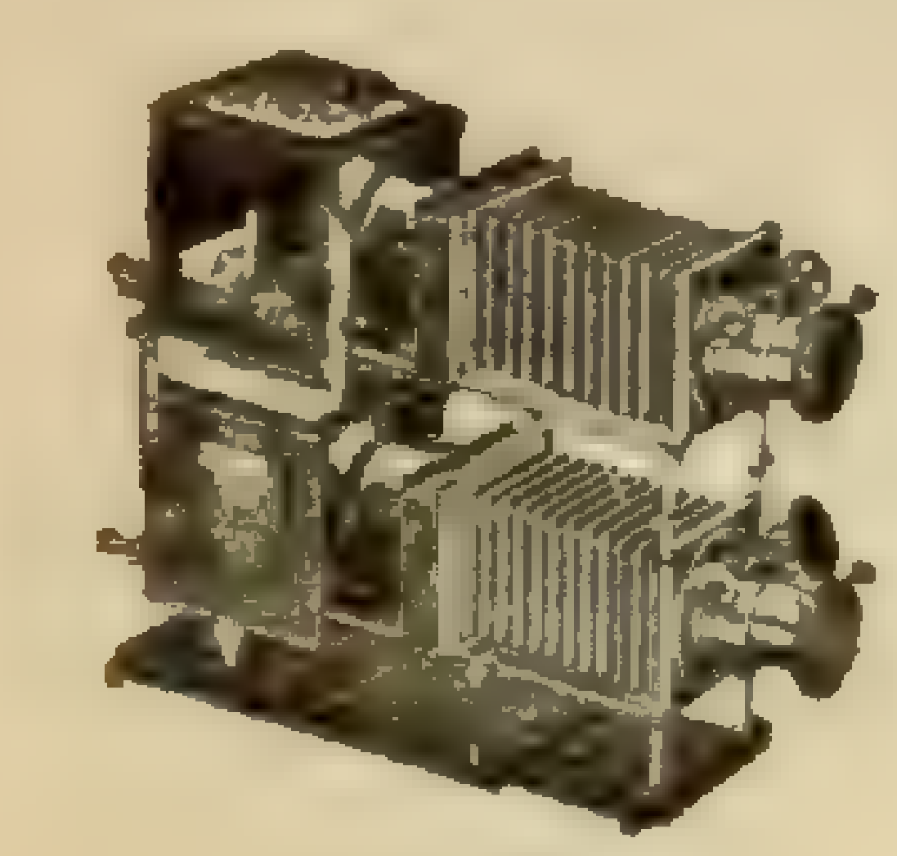

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VOLUME XXVIII NUMBER 5

BOTANICAL GAZETTE

NOVEMBER 1899

ON THE TOXIC EFFECT OF DELETERIOUS AGENTS ON THE GERMINATION AND DEVELOPMENT OF CERTAIN FILAMENTOUS FUNGI.[1]

J. F. CLARK.

INTRODUCTORY.

THE object of the writer in undertaking the investigation, the results of which are presented in this paper, was to determine approximately the relative and absolute toxic properties of a few deleterious agents as shown by their influence on the spores, the mycelium, and the fructification of certain of the mold fungi.

In recent years the study of plant pathology has come to be one of the most important in the whole range of botanical research. It seems desirable, therefore, that all possible light should be thrown upon the toxic properties of the various agents used in combating fungus pests. It is also very desirable, from a scientific point of view, to throw all light possible upon the problem as to the *element* or *group of elements* to the influence of which the toxic properties of the compound are to be attributed.

Thanks to the progress of modern physical chemistry, plant physiologists are now enabled to make up solutions of all chemical agents having the same number of molecules present in equal volumes of the various solutions. This is a decided step in advance, inasmuch as it enables investigators to compare the properties of a molecule of any substance with those of any other molecule, a comparison obviously impossible under the old method of making up solutions of a certain per cent. by weight. The vital error in making a comparison between percentage solutions is readily seen when we recall that a 1 per cent. solution of formaldehyde contains over eight times as many molecules per cc. as a 1 per cent. solution of mercuric chloride.

---

[1] A thesis presented to the Faculty of Cornell University for the degree Master of Arts.

These equi-molecular solutions are made by dissolving as many grams of the compound as there are units in its molecular weight in 1000 grams of the solvent. Such a solution is termed a "*normal solution*" of that compound, and it is represented conveniently by the formula $\frac{n}{1}$. Diluted to half this strength we get $\frac{n}{2}$, or one half normal solution, and so on. Solutions stronger than normal may be made by using double or quadruple the number of grams specified to the 1000^{cc} of the solvent, giving $\frac{2n}{1}$ and $\frac{4n}{1}$, respectively, of the substance.

Still more recent chemical and physical research has shown that in the case of very many substances in solution, many of the molecules of the dissolved substance are no longer present as such, but have become divided into two or more parts. These part-molecules have been termed *ions*. To illustrate, let us suppose that 36.37 grams of pure HCl have been added to 1000 cc. of water; we know that the HCl is no longer all present as whole molecules. It fact, we have every reason to believe that about 80 per cent. of it has become *ionized* into $\overset{+}{H}$ and $\overset{-}{Cl}$ ions, the $\overset{+}{H}$ ions being charged with positive electricity, the $\overset{-}{Cl}$ ions having a corresponding negative charge. The ions of all substances capable of ionization are similarly charged, and have been named *cathions* and *anions*, respectively, from the fact that those charged with positive electricity (cathions) migrate towards the cathode when a current of electricity is passed through the solution, while those charged with negative electricity migrate in the opposite direction towards the anode. As we have already said, a $\frac{n}{1}$ solution of HCl is about 80 per cent. ionized. This percentage of ionization increases with each increase of dilution, becoming practically complete at $\frac{n}{1000}$ in the case of HCl. Limited space forbids further discussion of this interesting and important phenomenon. For further information the reader is referred to the excellent papers by Drs. Kahlenberg and True ('96) and Mr. F. D. Heald ('96), or to the more recent text-books on physical chemistry. Suffice to say in this place that the ionization of the molecule has enabled chemists and physiologists to determine in many cases the *toxic element* or *group* in poisonous compounds. We shall have occasion to refer frequently to this matter in discussing the experimental data presented in this paper.

It has been the writer's aim to supplement the work of Drs. Kahlenberg and True, and Heald on phanerogams, and Drs. Krönig and Paul ('97) on bacteria, by applying the theory of the ionization of the molecule to the study of the physiology of fungicides.

METHODS.

Selection of forms.—In the selection of forms the following points were given particular consideration: (*a*) *regularity of germination*, (*b*) ability to grow normally in liquid media, (*c*) ability to fruit normally in a saturated atmosphere. After experimentation with a large number of forms the following were chosen as being well suited for the work: *Aspergillus flavus* Link, *Sterigmatocystis nigra* v. Tieghem, *Œdocephalum albidum* Saccardo, and *Penicillium glaucum* Link. *Botrytis vulgaris* Fr. was afterwards chosen as a fifth form, it being entirely satisfactory in regard to germination and mycelial development, and especially desirable because of its semi-parasitic habit; it, however, failed to fruit in cell cultures. Pure cultures of these molds were obtained and renewed from week to week. The spores used in inoculating the cultures in the experimental work were taken from fresh tubes in which the fungus had been growing seven to fourteen days. A solid medium made by adding 12^{gm} of agar to a liter of sugar beet infusion was found to be very satisfactory for stock cultures.

Selection of medium.—The object being to test the effect of the deleterious agents on the fungi under as nearly normal conditions of development as possible, the selection of a suitable medium was of primary importance. Many preliminary cultures were made with various media, including distilled water, infusions of potato, celery, sugar beet, prune, and bean (stems, pods, and mature seeds), besides various others compounded from inorganic salts, sugars, asparagin, etc. The results in germination and development were very varied. In distilled water Sterigmatocystis and Penicillium failed to germinate in 24 hours at 28° C., and of Botrytis, which did the best of the five forms, but 40 per cent. germinated in that time. Mycelial development in all was meager, and fruiting generally *nil.* Very minute quantities of the deleterious agents were found to inhibit germination, but the *death-point* of the spore was found in the case of dichloracetic acid, potassium hydroxid, and cobaltous sulfate, to be the same as in the medium finally selected. The media compounded from salts, sugars, etc., were more satisfactory; the vegetable infusions, however, were superior to all others. An infusion of sugar beet was ultimately chosen as being on the whole the medium best suited for the forms used. The fact that, in the case of the three typical poisons mentioned above, the concentration causing the death of the spore proved to be the same, whether the agent was dissolved in distilled water or the beet infusion, is very important in that it shows that this medium, which was used throughout the study, does not perceptibly change the toxic properties of these agents towards the fungus spores. The infusion of sugar beet was prepared by steeping 450 grams of the root in a liter of water for 3 hours at 100° C. It was then strained, cooled, and stirred up with the whites of two eggs, after which it was boiled, strained, filtered, and poured into flasks. After thorough sterilization the infusion was ready for use. In order to get the greatest possible uniformity it was found desirable to make up four or five liters at a time. In this medium the spores of all the forms used germinated quite uniformly in from 3 to 8 hours (according to the species), grew rapidly, and fruited normally (except Botrytis) in from 18 to 44 hours at a temperature of 28°C.

Method of culture.—The van Tieghem hanging-drop culture was found to be entirely satisfactory. The cylinder part, which was of glass, had an internal diameter of 17.5^{mm}, and a height of 10.7^{mm}. This cell, with a capacity of over 2.5 cubic centimeters, provided an abundance of oxygen for the normal development of the fungi. The cylinders were cemented to the slip by means of beeswax, and the cell was completed by sealing the cover to the top of the cylinder by means of a ring of vaseline, applied by inverting it on a glass slip covered by a thin layer of melted vaseline. A small nick was made in this ring so that when the cover was applied a minute opening might be left through which the expanding air could pass when the cultures were placed in the thermostat. An hour later they were carefully examined, and such as had not already become hermetically sealed were now made so by tapping with a pencil over the tiny opening referred to above. This precaution prevents much trouble and loss when culturing with volatile substances. For convenience in handling and examining under the microscope two cells were placed on each slip. The cells were permanently labeled by gumming lettered and numbered labels to the ends of the slips. As a precaution against accidents duplicate cultures were not placed on the same slip.

The various dilutions in beet infusion of the compound to be tested were made up in a dozen or more bottles of about 30^{cc} capacity. Each bottle was provided with a glass rod, drawn to a blunt point, by means of which the culture drop was transferred to the cover. *Four or five drops of the same solution were then placed in the bottom of the cell.* The spores of the fungus to be tested were transferred from a pure culture to the hanging-drop by means of a sterile platinum needle, the utmost care being taken to *prevent the adherence of the spores in bunches* in making the inoculation. The cover bearing the culture was then inverted on the cell and gently pressed until completely closed, except for the minute opening already fully described. When a set of cultures was complete all were placed in a thermostat which was kept at a constant temperature of 28°C.

Care of cells, covers, pipettes, etc.—After completion of a series of cultures, all bottles, rods, etc., were thoroughly washed and placed in running water for several hours, then dried and placed in a dry oven at 160°C. When the cultures in the cells matured, the covers were removed and the cells were thoroughly washed under the water tap, wiped, air-dried, and finally placed in the dry oven at 110°–120°C. for an hour. This insured thorough sterilization, and at the same time drove off the last trace of any volatile substance that might have escaped the washing. The covers were first boiled in strong KOH, then in several changes of water; this was followed by boiling in strong $H_2SO_4 + K_2Cr_2O_7$. They were again thoroughly rinsed and again boiled in four changes of clear water, rinsed in 95 per cent alcohol, wiped, and sterilized at 160°C. The pipettes were cleaned by forcing water through them for an hour by attaching to the water tap. They were then sterilized in a steam sterilizer.

An occasional culture was found to be contaminated with bacteria, due no doubt to dust particles bearing spores coming in contact with the cultures in the making. Such contamination, however, by bacteria or fungi amounted to less than 1 per cent of the cultures made after the completion of preliminary experimentation.

Vapor pressures in the cell.—It has long been known to physical chemists that every liquid has a certain vapor pressure. Pure water at standard atmospheric pressure and 28°C., has a vapor pressure of 28^{mm} of mercury. Any addition of a substance or substances to this water will lower its vapor pressure. If the substance added be hygroscopic the lowering of the vapor pressure may be very great, as is the case with KOH and H_2SO_4. On the other hand, should the substance be quite volatile the increased vapor pressure of the substance added may more than counterbalance the lowering of the vapor pressure of the water, as is the case when ammonia or alcohol is mixed with water. To recapitulate in brief, the addition of any substance or substances to water gives a mixture with a vapor pressure at variance with that of pure water. This vapor pressure may be greater or less than that of pure water, depending on the physical properties of the substance or substances added. Thus, a hanging-drop containing KOH in a cell in which water has been placed below (as is the usual method), absorbs moisture by reason of its low vapor pressure. Indeed, there will be a constant distillation of water vapor from the water below to the culture drop, until all the water has passed up or the drop, becoming too large to "hang," falls to the bottom of the cell. With alcohol the reverse takes place. No sooner is such a culture made up than the alcohol begins to distill from the hanging-drop, and it has not, even for an hour, the concentration supposed to be present. Such a culture as a test of toxicity is valueless.

Were it possible to have all hanging-drops of exactly the same size, and exactly the same quantity of water below, we should expect uniformity in results. Such uniformity, however, would be useless, perhaps worse than useless, as it might prevent attention being called to the fundamental error of the method, viz., the use of solutions of varying vapor pressures in the same cell. As a matter of practical experience, however, it is impossible to have all hanging-drops of exactly the same size, and erratic germinations are consequently the inevitable result. To quote from one of the more recent studies, Stevens (9) found that of four cultures of Macrosporium in a $\frac{n}{819200}$ solution of mercuric chlorid, three grew and one failed; of eight cultures in $\frac{n}{409600}$ five grew and three failed; of ten cultures in $\frac{n}{204800}$ four grew and six failed; of four cultures in

$\frac{n}{102400}$ one grew and three failed! With a strictly non-volatile substance we should expect less variation, because with less difference between the vapor pressures of the hanging-drop and that of the water below, the changes in the concentration of the deleterious agent in the hanging-drop would necessarily take place more slowly. Of the volatile properties of $HgCl_2$ we shall speak later.

In looking for a method that would meet every requirement of the case, many preliminary tests were made with all five molds and with different deleterious agents. Two such tests with potassium cyanid are here presented in detail. It is of interest to note that while KCN is not in itself a volatile compound, in aqueous solution more or less hydrolysis takes place, resulting in the formation of a corresponding amount of HCN (Shields '93), which is quite volatile. Hence, aqueous solutions of KCN are in their behavior quite typical of volatile compounds.

Column 1 gives the culture label by means of which the cultures were identified; column 2 the concentrations of KCN in the hanging-drops of the various cultures; column 3 the solution—if any—used in the bottom of the cell. "Dry" implies that no water or other solution was placed in the bottom of the cell. Under the head "germination" the percentage of spores germinated is given for three observations, at 12, 24, and 36 hours, respectively. Under "development" the length of the germ tubes of spores showing an average development is given in micromillimeters.

These data not only show that cultures having *water* in the bottoms of the cells are unreliable and vary according to the *amount* of water present, but that "dry" cells are equally unsatisfactory. Especial attention is called to the record of the cultures labeled "A10." In these the culture drop contains but one fourth of the KCN present in cultures labeled "A9," the former, however, had several drops of the solution from which the hanging-drop was made placed in the bottom of the cell instead of the customary drop of water. The striking difference in the results

ASPERGILLUS IN BEET INFUSION + KCN

Temperature 28° C.

Culture label	Str'ngth of solution	Conditions of cell	Germination			Development			Notes
			12 hrs.	24 hrs.	36 hrs.	12 hrs.	24 hrs.	36 hrs.	
			%	%	%	μ	μ	μ	
A1	–KCN	(Check culture)	100			250	∞	∞	Fruited in 20 hrs.
A7	$\frac{n}{32}$	Dry	25	100		barely germ.	45	200	
A7	$\frac{n}{32}$	"	10	100		"	40	100	
A8	$\frac{n}{32}$	One drop of water	7	100		"	120	500	
A8	$\frac{n}{32}$	" "	5	100		"	100	800	
A9	$\frac{n}{32}$	Cell half filled with water	45	100		140	∞	∞	Well fruited at 38 hrs.
A9	$\frac{n}{32}$	" " " "	70	100		150	∞	∞	" "
A10	$\frac{n}{128}$	$\frac{n}{128}$ KCN solution	0	0	75	..	..	100	
A10	$\frac{n}{128}$	" "	0	0	10	..	..	80	

PENICILLIUM IN BEET INFUSION + KCN

Temperature 28° C.

Culture label	Str'ngth of solution	Conditions of cells	Germination			Development			Notes
			12 hrs.	24 hrs.	36 hrs.	12 hrs.	24 hrs.	36 hrs.	
			%	%	%	µ	µ	µ	
E1	-KCN	(Check culture)	100			110	∞		Fruited at 24 hrs.
E7	$\frac{n}{32}$	Dry	0	5	10	...	barely germ.		No development
E7	$\frac{n}{32}$	"	0	3	10	...	"	70	
E8	$\frac{n}{32}$	One drop of water	.1	10	25	barely germ.	20	150	
E8	$\frac{n}{32}$	" "	.01	10	20	"	20	130	
E9	$\frac{n}{32}$	Cell half filled with water	2	70		8	80	∞	Fruited at 36 hrs.
E9	$\frac{n}{32}$	" " " "	10	75		15	100	∞	" "
E10	$\frac{n}{64}$	$\frac{n}{64}$ KCN solution	0	0	0	...	...	...	
E10	$\frac{n}{64}$	" "	0	0	0	...	...	..	

needs no further comment. The results with Penicillium and other forms abundantly confirmed the test, and lead to the conclusion that *when water is used at the bottom of cells containing hanging-drop cultures and solutions of substances having very high vapor pressures are being tested, erratic germinations are to be expected, and in the case of a test of any substance—particularly those of high or low vapor pressure—the toxic properties shown will be less than a correct method would indicate.*

In the regular work reported in this paper, a few drops of the same solution as that used in the hanging-drop of any particular cell was in every case placed in the bottom of that cell, thus establishing complete equilibrium of vapor pressures in the cells, and thereby preventing changes in the concentration of the solution under test as must take place when this precaution is not observed. By this method it was found quite feasible to make hanging-drop cultures of any composition whatever, ranging from a $\frac{16n}{1}$ (70 per cent.) solution of alcohol to a $\frac{4n}{1}$ (21 per cent. solution of potassium hydroxid.

Stock solutions of chemicals.—These were prepared in all cases by a responsible chemist from the purest chemicals obtainable. It was found desirable to have stock solutions in highly concentrated form so that all necessary diluting could be made by adding beet infusion. The stock solution of HCl, for example, contained 25 grams pure HCl per 100^{cc}. $\frac{n}{1}$ HCl contains 3.58 per cent. HCl by weight. Such a solution was gotten by taking 5^{cc} of the stock solution and diluting, by adding beet infusion, to 34.9^{cc}. $\frac{n}{2}$ was gotten by diluting 10^{cc} $\frac{n}{1}$ to 20^{cc}, and so on. Chemical agents liable to deteriorate in quality (*e. g.*, KCN) were titrated by the chemist on the morning of the day on which cultures with it were made up.

Trial and regular cultures.—Inasmuch as the work was in most cases of a pioneer character, it was found desirable to make up a large number of trial cultures preliminary to the regular work in order to get some definite idea as to the toxicity of the various chemicals to this group of plants; for it was of course early learned that the data worked out for phanerogams by Kahlenberg and True ('96) and Heald ('96) were a contrast rather than a comparison when placed beside the facts learned by experiment from the fungi. Heald ('96) called attention to this fact on finding a fungus growing vigorously in a solution of HCl that had killed the root of Pisum. The data obtained from the trial cultures proved invaluable in making up the regular cultures, as it was then possible in most cases to make up a series of twelve dilutions in no. 1 of which the spores would certainly be killed, while in no. 12 the fungus would be practically unharmed.

In the regular work all cultures were made up in duplicate, including duplicate checks in pure beet infusion. Important points were quite frequently checked over in duplicate and sometimes in quadruplicate. This was also done in the case of *unexpected* developments; for instance, the writer was surprised to find the spores of Penicillium showing so great a specific resistance to acetic acid. Repeated checking, however, proved the correctness of the first observation.

One of the most marked features of the entire work was the *regularity in results.* It is true that the cultures nearest the inhibiting point in a few cases varied to the extent that germination of a number of spores took place in one culture while the duplicate failed. Such were not considered *erratic.* If, however, both cultures of a certain strength grew and one of some weaker concentration failed, the latter would be considered erratic. Such erratic cultures, however, did not exceed a dozen in a work requiring upwards of forty-five hundred regular cultures aside from preliminary work. Such as did occur were doubtless due to oversight in cleaning the cells or other accident.

Examination of cultures, noting results, etc.—Cultures were made up in the early part of the day, and were examined at intervals of from three to six hours for the fourteen hours following, and at longer intervals until the fungus had matured, or the spores in the cultures which had failed to germinate were transferred to pure beet infusion to test their vitality. The percentage of spores germinated in the various cultures was noted on two occasions, first when from 30 per cent. to 70 per cent. of those in the checks had germinated, and again a few hours later. A similar method was adopted to indicate the early mycelial development. At some time after germination the germ tubes showing an average development were measured and noted; this was repeated a few hours later, and sometimes a third measurement was made. The first appearance of conidia was also noted, but as this very frequently occurred at night (between 15 and 22 hours after inoculation) the point was not so well noted.

In the earlier work, the cultures in which the spores *failed to germinate* were opened at 72 hours and a number of the spores were transferred by means of a sterile platinum needle to a hanging-drop of pure beet infusion in a clean cell, in order to test whether they were killed by the agent or merely inhibited. If 1 per cent. or more survived, the culture was classed as *inhibited;* if none at all or less than 1 per cent. survived, they were classed as *killed.* In most of the work, however, the transfers were made at 48 hours, it being found that all spores that could germinate did so in less than 36 hours and usually much less.[1]

The cultures which germinated in the presence of the deleterious agent were likewise divided into two classes: (1) those which although they may have been retarded or stimulated in mycelial development by the agent, finally matured a fair crop of conidia in about the normal time; (2) those, which, although they germinated and continued to grow, presented a markedly irregular or retarded mycelial development, and generally failed to fruit. Between these two classes came—as might be supposed—a number of cultures which were very difficult to classify. In some cases there would be an apparently normal mycelial development but almost total supression of fruiting. In other cases an irregular, meager, and even yeast-like mycelium would cause surprise by finally developing a number of apparently normal fruits. In general, a fungus was said to be "injured" by that concentration of the deleterious agent which prevented its classification in class I as above.

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[1] Cultures in alcohol, formaldehyde, H_2O_2, and KCN were transferred at 72 hours, all others at 48 hours.

In discriminating between class III (inhibited spores) and class IV (killed spores) attention is again called to the importance of avoiding *bunches* of spores in making the inoculation. In many solutions such bunches — doubtless containing air — float on the surface of the drop and fail to receive the full influence of the agent. When spores from such a culture are transferred to pure beet infusion to test their vitality the bunch may be broken up in the process and the spores germinate readily while all others are dead. Much can be done with care in making the inoculation, but at best it is a serious source of error, and it has been on this account, and on account of the impossibility of transferring the spores without taking with them small quantities of the agent, that the *inhibiting concentration* rather than that causing death has been adopted as the *chief critical point* in discussing the experimental data.

To illustrate what has been said regarding the taking of notes on cultures, a typical left-hand page of the culture notebook is here reproduced. This will also aid the reader to understand the classification of the experimental data presented later. The opposite page was always reserved for more extended notes on points observed from time to time in the progress of the experiment.

Sources of error.—Before proceeding to a discussion of the experimental data it might be well to mention briefly the sources of error observed during the preliminary study and guarded against in the progress of the work.

1. Xylonite cells were found to have an injurious influence on some fungi when used for hanging-drop cultures at 28°C.

2. Bacterial contamination.

3. Lack of equilibrium in vapor pressures in the cells.

4. Deterioration of stock solutions (see details of experiment with KCN).

5. Use of impure vaseline for sealing cells.

ASPERGILLUS IN BEET INFUSION + $CoSO_4$.

Cultured at 9 a. m., Feb. 21, 1899. Temperature, 28° C.

Chart	No. of culture	Culture label	Strength of solution	Germination				Mycelial development						Fruit hrs.	Notes
				hrs.	%	hrs.	%	hrs.	μ	hrs.	μ	hrs.	μ		
	2479	A1	—$CoSO_4$	6	85			6	30	12	200	22	∞	22	Record of duplicates omitted to save space
	2481	A2	$\frac{n}{2048}$	6	80			6	30	12	210	22	∞	22	
I.	2483	A3	$\frac{n}{1024}$	6	85			6	30	12	200	22	∞	22	
	2485	A4	$\frac{n}{512}$	6	80			6	30	12	180	22	∞	27	
	2487	A5	$\frac{n}{256}$	6	75	8	100	6	25	12	170	22	∞	27	
Intermediate	2489	A6	$\frac{n}{128}$	8	50	12	100			12	60	22	∞	36	
	2491	A7	$\frac{n}{64}$	12	1	22	100					22	170	48	Well fruited at 72 hrs.
II.	2493	A8	$\frac{n}{32}$	22	1	36	2					22	15	72	Few fruits only
III.	2495	A9	$\frac{n}{16}$	[Trans	ferred	at 48	hrs. 12	hrs. 5%	germ.;	24 hrs.	many	more.]			
	2497	A10	$\frac{n}{8}$	[Trans	ferred	at 48	hrs.	Failed.]							
Class IV.	2499	A11	$\frac{n}{4}$	"	"	"	"	"							
	2501	A12	$\frac{n}{2}$	"	"	"	"	"							

6. Use of spores of uncertain age and vitality.

7. Use of culture media unsuited for the normal development of the fungi tested.

8. Imperfect sealing of the cells, due generally to the raising of the cover by expansion of contained air when the cultures were placed in the thermostat.

9. Transference of bunches of spores in making inoculations.

EXPERIMENTAL DATA AND DISCUSSION.

Details regarding the critical points (*i. e.*, concentrations causing injury, inhibition, and death) of the various fungi in the more important of the thirty-seven compounds tested may be found by referring to the diagrams prepared to accompany this paper. This device, primarily intended to conserve space by giving in a condensed form the various specific resistances of the different molds will also be useful, it is hoped, in conveying to the reader, by means of the eye, a general impression as to the relative toxic properties of the agents tested. It should be distinctly borne in mind in consulting these diagrams that each vertical line represents a *doubling* of the number of molecules present in the solutions in passing toward the right, the whole space between two vertical lines representing one concentration. The relative *average* toxic properties of the more poisonous agents are further graphically depicted by means of the two charts in the final installment of this paper.

ACIDS.

In the case of acids, diagrams have been prepared, giving the resistances of the individual molds in the eight acids. These diagrams will be found to accompany the diagrams for the various acids on pages 307–308. In these diagrams an attempt has been made to emphasize the fact that the solutions double in concentration in passing to the right, by placing at the head of the columns the proportions of molecules present in each solution in terms of x; x being in every case the number of molecules present in a $\frac{n}{262144}$ solution. Thus a normal solution always contains $262144x$ molecules, $\frac{2n}{1}$ contains $524288x$ molecules, and so on.

Hydrochloric acid, HCl; 70, *230*, 614. This acid, on account of its very high ionization at the critical points, and because of its very wide use in physiological and chemical investigations by other workers, has been taken as a *standard* by which we shall compare the others. In making these comparisons, HCl, being the most highly ionized of all acids, is *assumed* to represent the value of ionic $\overset{+}{H}$. This is a purely arbitrary assumption, and the reader will bear in mind that when we speak of "the toxic value of ionic $\overset{+}{H}$" we mean the nearest approach we could make to determining its toxic value for the molds, viz., *that of 91% ionic* $\overset{+}{H}$ *+9% HCl.*

The following "coefficients" have been worked out for HCl with these five molds: coefficient of injury, 70; coefficient of inhibition, *230;* coefficient of death-point, 614.

These coefficients mean that *on an average for the five forms used* $\frac{70}{2048}$ of a normal solution caused distinct injury to the cultures, $\frac{230}{2048}$ of a normal solution inhibited the germination of the spores, and $\frac{614}{2048}$ of a normal solution killed the spores. The denominator of the fractions, 2048—the eleventh power of 2—has been used throughout this paper in determining coefficients. As a matter of convenience the numerator only has been expressed in discussing the three critical points of the various agents, giving as it does at a glance the correct *relative* toxicity of the agents. The *absolute* value of any coefficient may (in either mold or agent) be determined by simply supplying the omitted denominator, 2048, the resulting fraction being in every case that proportion of a normal solution. In discussing the various agents these coefficients will be placed immediately after the chemical formulæ in the order given. That expressing the inhibiting value is *italicized* to emphasize the fact that it is regarded as the most significant.

From time to time attention will be called to the wonderful resistance of the fungi to many deleterious agents as compared with that of the higher plants. The comparison (or contrast) in resistance to ionic $\overset{+}{H}$ is as 800 : 1, the average death-point for the five species of molds being $\frac{n}{3 \cdot 3}$ HCl, while that of three species of phanerogams is $\frac{n}{2740}$ HCl. (Heald, '96, p. 152.)

On the whole, HCl was at the same time the most completely ionized and the least toxic of the acids tested. Sterigmatocystis proved most resistant, for although germination and early mycelial development were distinctly retarded by $\frac{n}{1024}$, $\frac{n}{4}$ was required to inhibit all the spores, and $\frac{n}{2}$ for forty-eight hours to kill them. Cultures in $\frac{n}{16}$, although much retarded in early development, had at forty-eight hours quite overtaken the checks, and at seventy-two hours far surpassed them in amount of mycelium produced. Distinct retardation of fruiting was first noticed in $\frac{n}{128}$; $\frac{n}{16}$ required nearly double the time to mature its fruit as

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EXPLANATION OF DIAGRAMS.

The initials A., S., Œ., B., P. stand for the respective generic names of the fungi used.

The fraction of a normal solution placed at the head of a column refers to the *space between the vertical lines* over which it stands. In this space is depicted the result of culturing the various fungi in this concentration of the different agents by the symbols described below.

═ Two lines indicate normal or almost normal development.

☰ Three lines indicate distinct injury.

≣ Four lines indicate very great injury.

▮▯▮ Alternate blocks indicate total inhibition of germination.

■ Solid black indicates death of spore when tested for vitality at 48 hours.

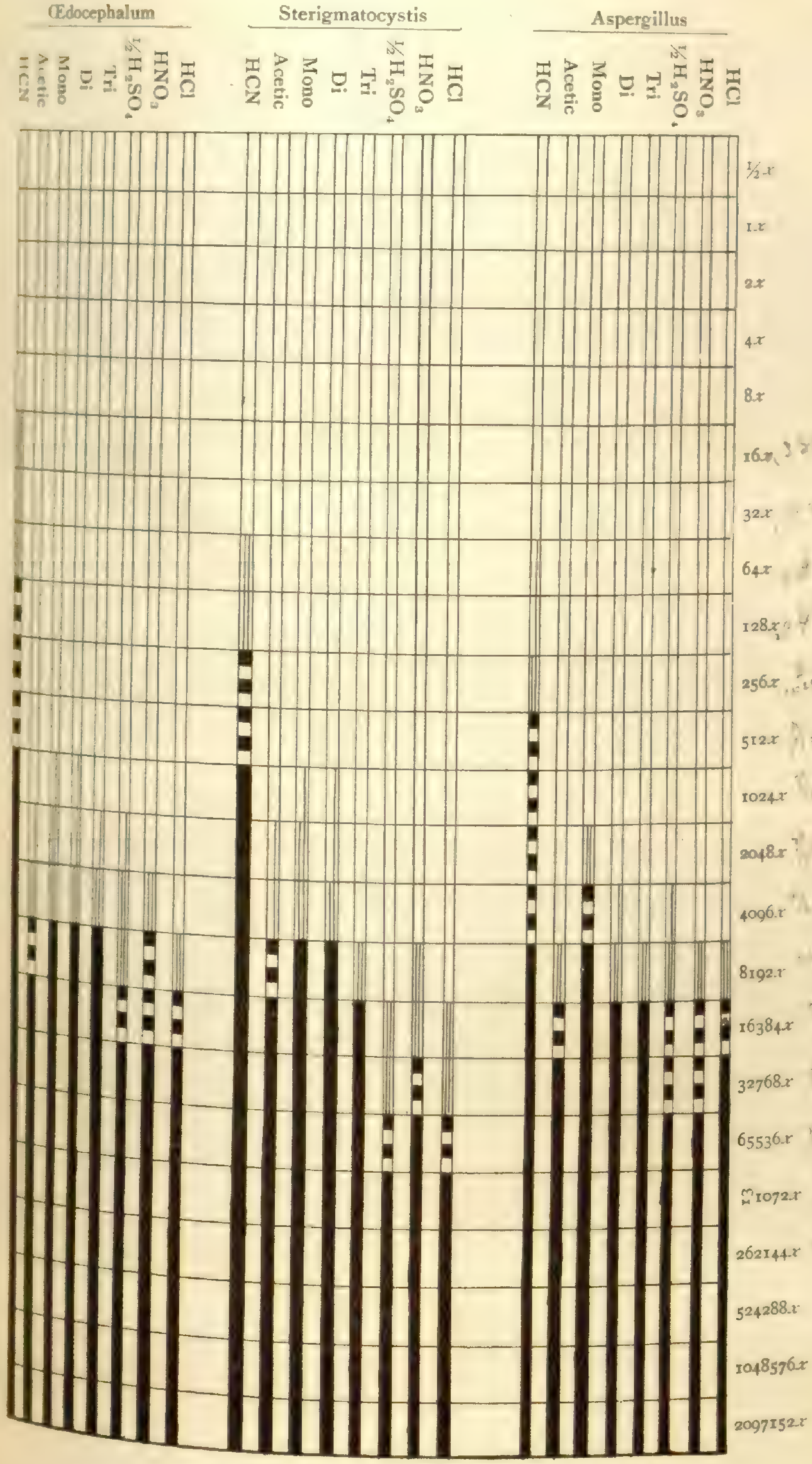

Œdocephalum
HCN
Acetic
Mono
Di
Tri
$\frac{1}{2}H_2SO_4$
HNO_3
HCl
Sterigmatocystis
HCN
Acetic
Mono
Di
Tri
$\frac{1}{2}H_2SO_4$
HNO_3
HCl
Aspergillus
HCN
Acetic
Mono
Di
Tri
$\frac{1}{2}H_2SO_4$
HNO_3
HCl
½x
1.x
2.x
4.x
8.x
16.x
32.x
64.x
128.x
256.x
512.x
1024.x
2048.x
4096.x
8192.x
16384.x
32768.x
65536.x
131072.x
262144.x
524288.x
1048576.x
2097152.x

DIAGRAM I

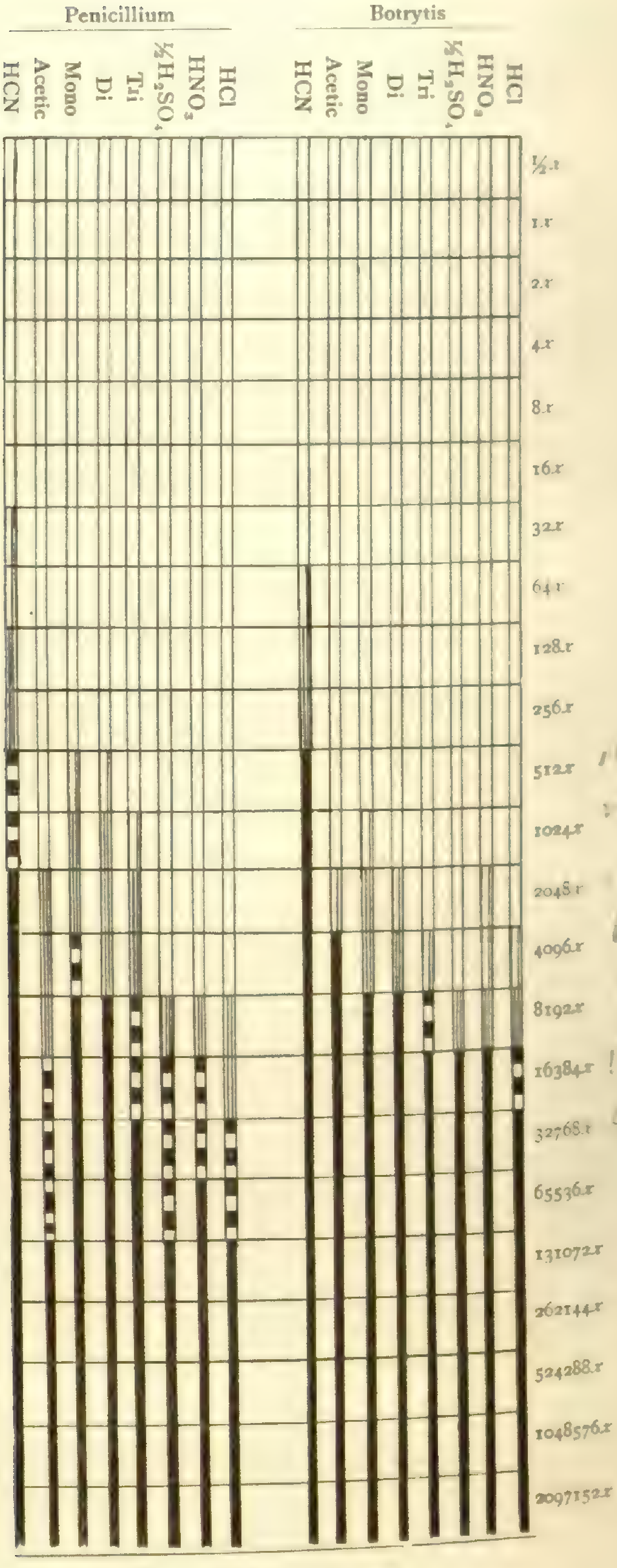
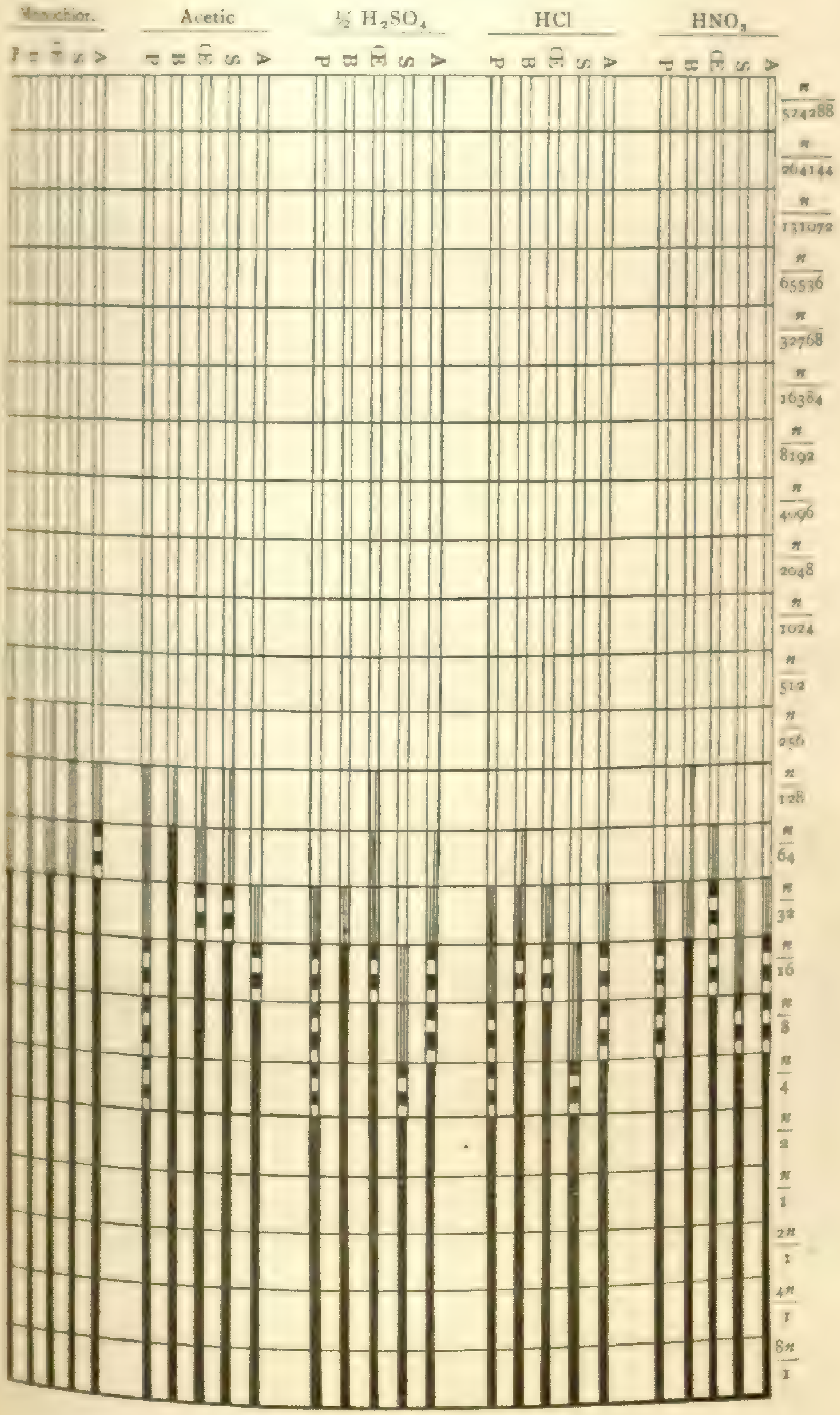

Penicillium
Botrytis
HCN
Acetic
Mono
Di
Tri
½H₂SO₄
HNO₃
HCl
HCN
Acetic
Mono
Di
Tri
½H₂SO₄
HNO₃
HCl
½.r
1.r
2.r
4.r
8.r
16.r
32.r
64.r
128.r
256.r
512.r
1024.r
2048.r
4096.r
8192.r
16384.r
32768.r
65536.r
131072.r
262144.r
524288.r
1048576.r
2097152.r

DIAGRAM II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Acetic
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HCl
HNO_3
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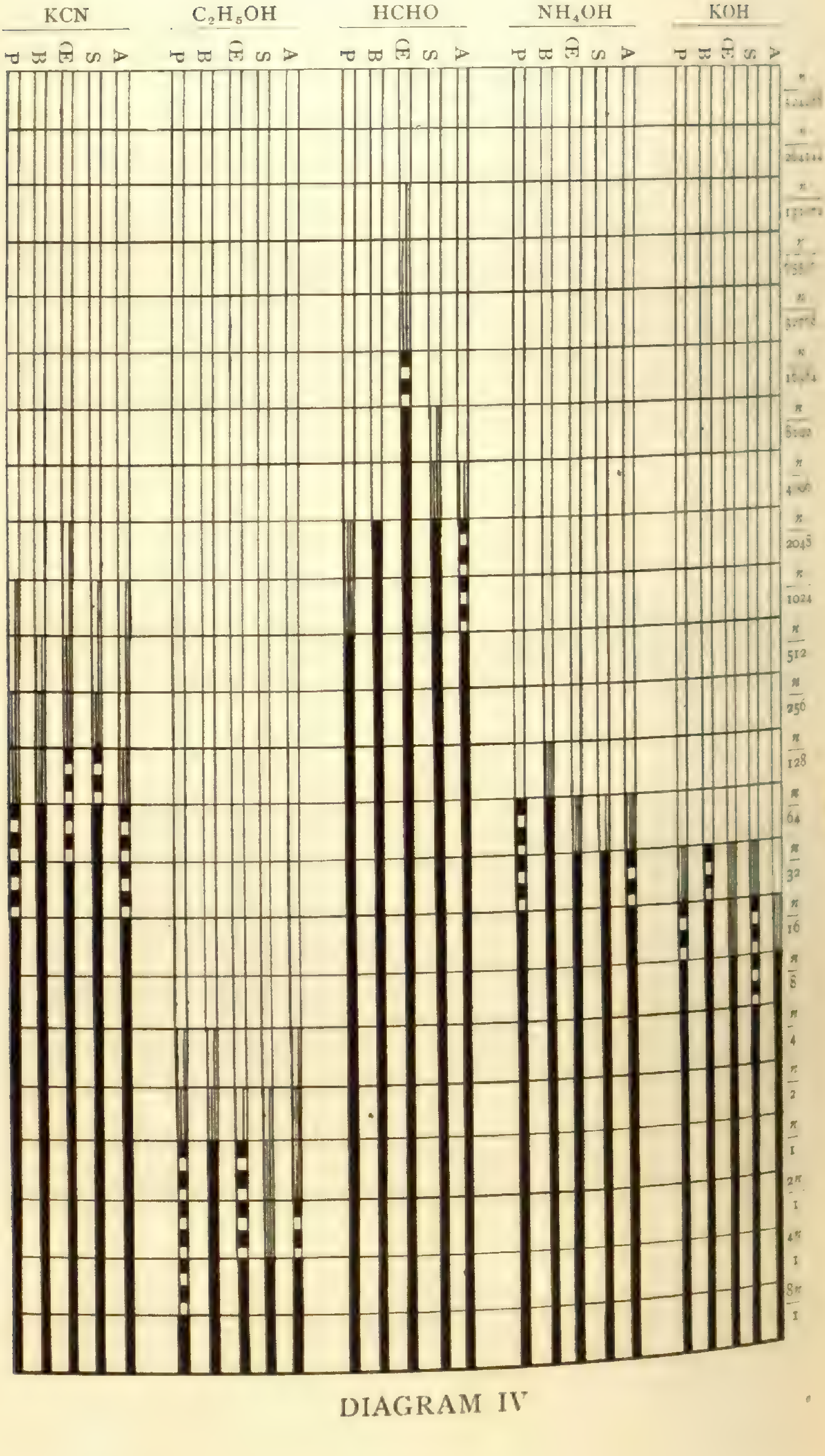
P B Œ S A
P B Œ S A
P B Œ S A
P B Œ S A
n/524288
n/264144
n/131072
n/65536
n/32768
n/16384
n/8192
n/4096
n/2048
n/1024
n/512
n/256
n/128
n/64
n/32
n/16
n/8
n/4
n/2
n/1
2n/1
4n/1
8n/1

DIAGRAM III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HCHO
NH$_4$OH
KOH
P
B
Œ
S
A
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n/16384
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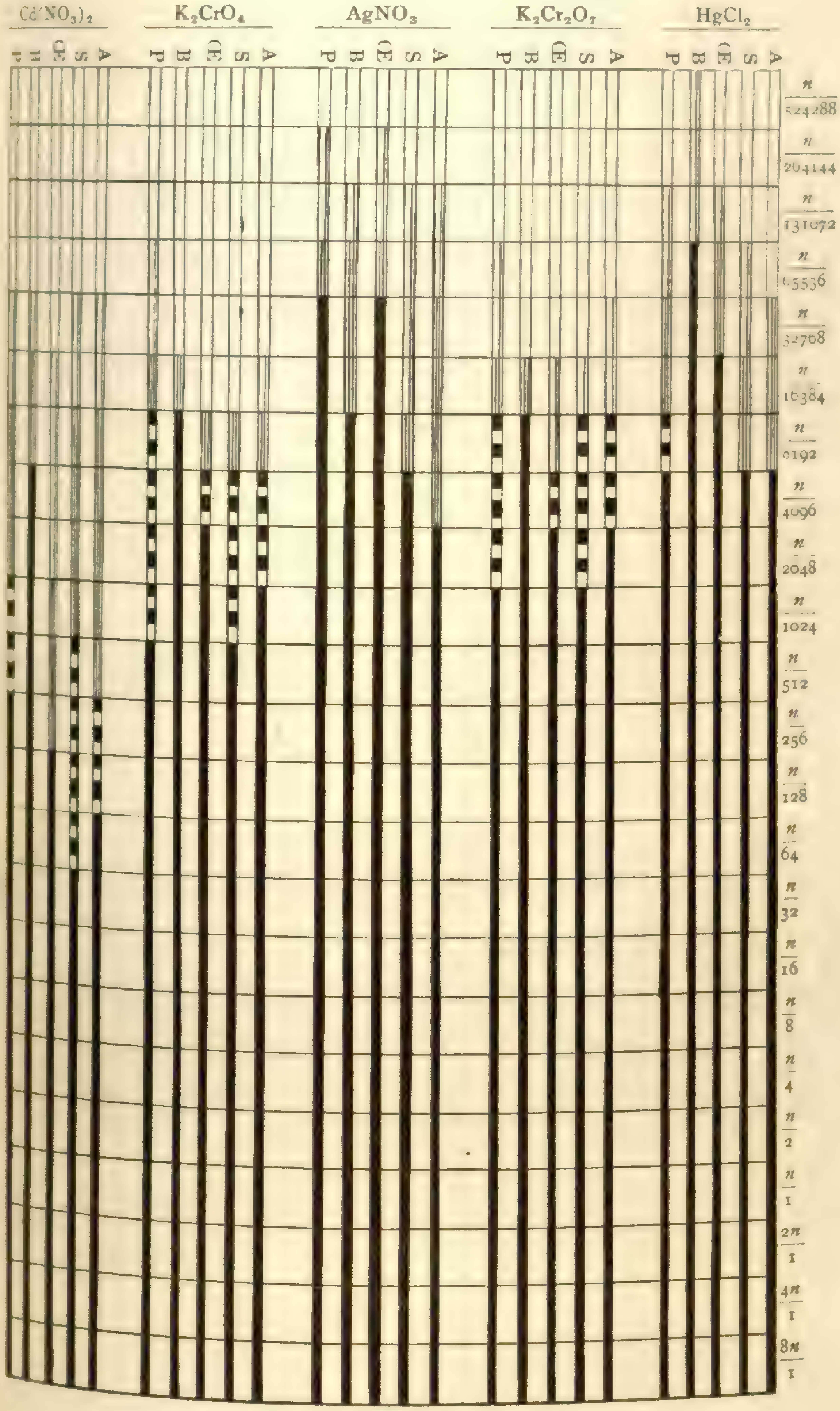
n/4096
n/2048
n/1024
n/512
n/256
n/128
n/64
n/32
n/16
n/8
n/4
n/2
n/1
2n/1
4n/1
8n/1

DIAGRAM IV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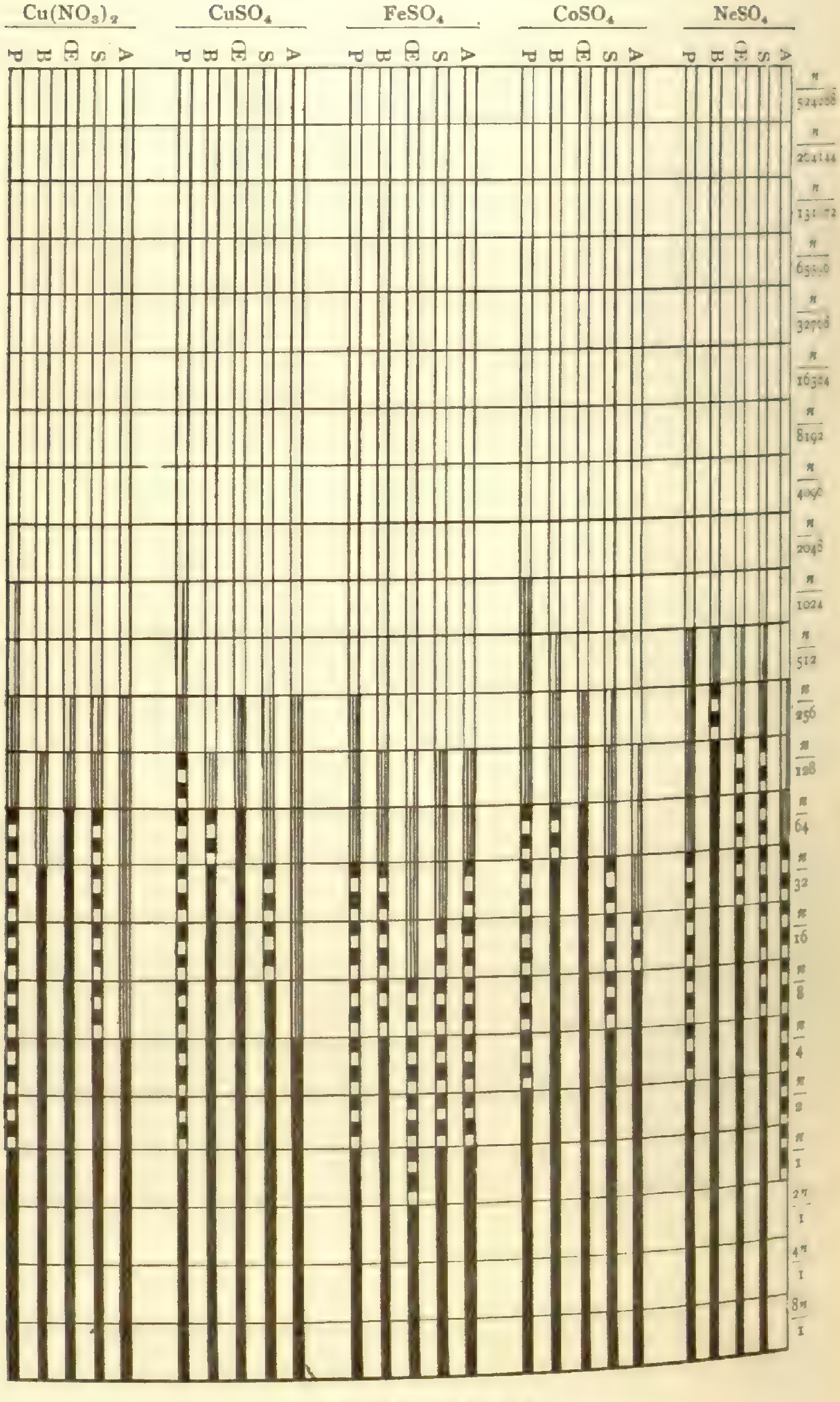
K₂CrO₄
P B Œ S A
AgNO₃
P B Œ S A
K₂Cr₂O₇
P B Œ S A
HgCl₂
P B Œ S A
n/524288
n/262144
n/131072
n/65536
n/32768
n/16384
n/8192
n/4096
n/2048
n/1024
n/512
n/256
n/128
n/64
n/32
n/16
n/8
n/4
n/2
n/1
2n/1
4n/1
8n/1

DIAGRAM V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CuSO4
FeSO4
CoSO4
NeSO4
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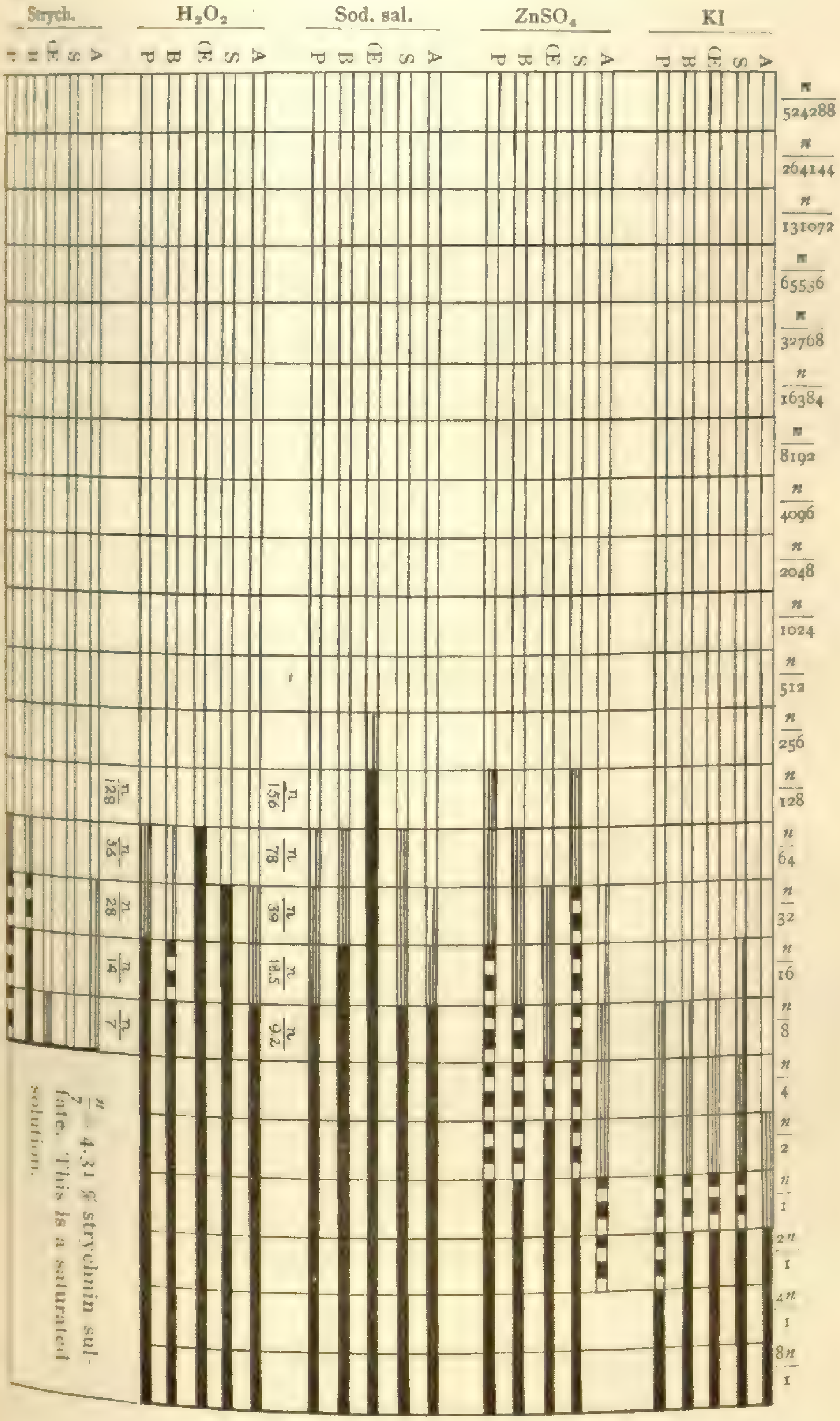
P B Œ S A
P B Œ S A
P B Œ S A
P B Œ S A
n/524288
n/262144
n/131072
n/65536
n/32768
n/16384
n/8192
n/4096
n/2048
n/1024
n/512
n/256
n/128
n/64
n/32
n/16
n/8
n/4
n/2
n/1
2n/1
4n/1
8n/1

DIAGRAM VI


Strych.
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H₂O₂
P B Œ S A
Sod. sal.
P B Œ S A
ZnSO₄
P B Œ S A
KI
P B Œ S A
n/524288
n/264144
n/131072
n/65536
n/32768
n/16384
n/8192
n/4096
n/2048
n/1024
n/512
n/256
n/128
n/64
n/32
n/16
n/8
n/4
n/2
n/1
2n/1
4n/1
8n/1
n/128
n/56
n/28
n/14
n/7
n/156
n/78
n/39
n/18.5
n/9.2
n/7 = 4.31 % strychnin sulfate. This is a saturated solution.

DIAGRAM VII

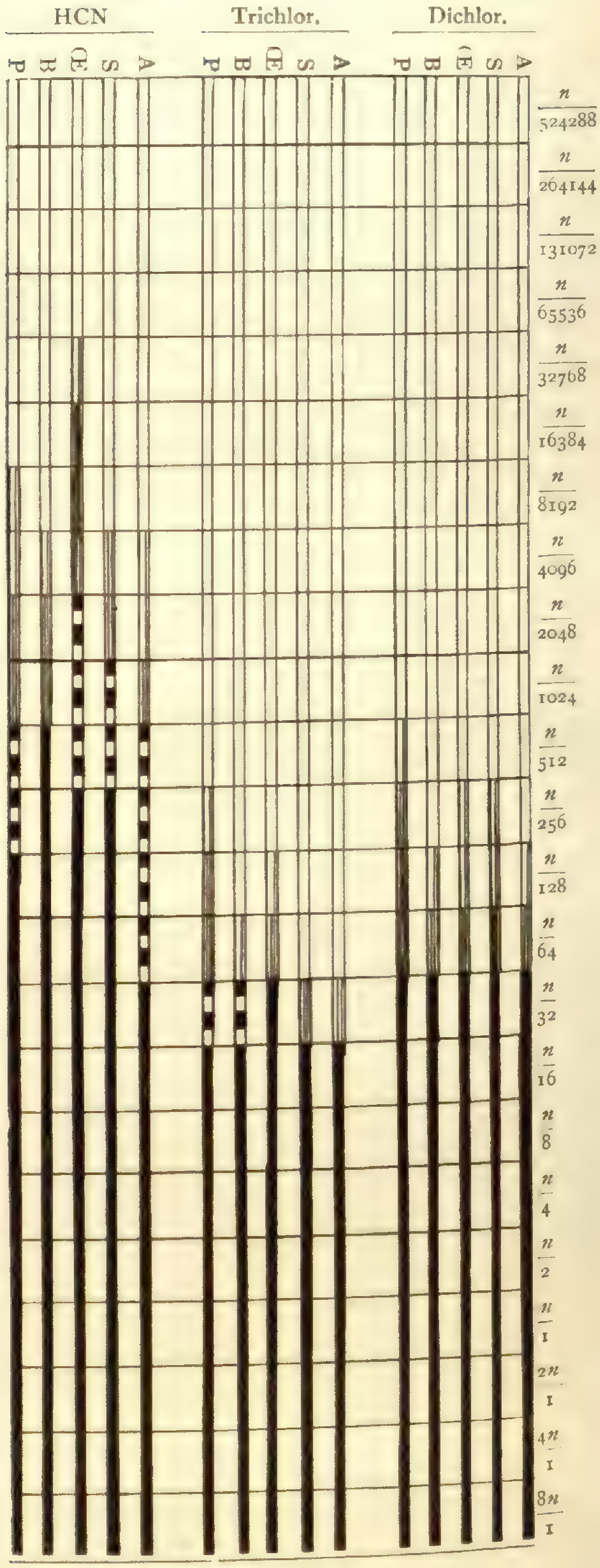

DIAGRAM VIII

compared with the checks; while $\frac{n}{8}$, the limiting culture, failed to fruit in six days.

With HCl a sixth form, *Rhizopus nigricans*, was tested. Like its near relatives the mucors, it makes very unsatisfactory growth in a liquid medium, and was therefore discarded. Its resistance to HCl proved to be practically the same as that of Œdocephalum. The limiting culture of Aspergillus, $\frac{n}{32}$, developed a number of abnormal conidiophores, each bearing 1–4 sterigmata, on the sixth day.

In the case of HCl, as in the case of all other acids tested, an attempt was made to determine the relative toxic value of the anion. The potassium salt was used. This salt becomes highly ionized into $\overset{+}{\mathrm{K}}$ and $\overset{-}{\mathrm{Cl}}$ ions in moderately concentrated solutions. In a $\frac{n}{4}$ KCl solution 84 per cent. of the salt is thus ionized. In a similar concentration of HCl 89 per cent. is ionized into $\overset{+}{\mathrm{H}}$ and $\overset{-}{\mathrm{Cl}}$ ions. (Kohlrausch, '85.) In the former we have $\overset{+}{\mathrm{K}}$ and $\overset{-}{\mathrm{Cl}}$ ions and some KCl; in the latter, $\overset{+}{\mathrm{H}}$ and $\overset{-}{\mathrm{Cl}}$ ions and some HCl. It is evident therefore that the essential difference between these solutions is the replacing of $\overset{+}{\mathrm{H}}$ in the latter by $\overset{+}{\mathrm{K}}$, and the HCl by KCl in the un-ionized portion. The significant point is that the *concentration of* $\overset{-}{\mathrm{Cl}}$ *ions is practically the same in both.* The $\frac{n}{4}$ HCl solution is fatal to the spores of Aspergillus, while $\frac{n}{4}$ KCl solution has practically *no injurious influence.* Indeed, this fungus germinates, grows, and fruits normally in a solution of double this concentration of KCl. Hence we know that the $\overset{-}{\mathrm{Cl}}$ ion is *relatively harmless* to this mold. Similar tests proved its low toxic value to all the molds used.

The absolute toxic value of ionic $\overset{-}{\mathrm{Cl}}$, and other weakly toxic anions, is, however, very difficult to determine. In this connection it should be remembered that the potassium salts of the various acids are, although highly, far from being completely ionized at the concentrations permitting germination of mold spores. Then, too, we are, perhaps, not justified in assuming that ionic $\overset{+}{K}$ is entirely non-toxic; for although potassium is a necessary food element for all plants, may it not be that great concentrations of even so good a thing as ionic $\overset{+}{K}$ may be bad for fungi? Later, this will be shown to be the case with iron, an element which, while absolutely necessary in small quantities for all plants (Molisch, '94), is quite toxic in excess for both fungi and higher plants.

Experiments with Aspergillus and Œdocephalum in KCl solutions, however, enable us to say quite positively that for these forms the anion $\overset{-}{Cl}$ has at most *less* than one thirty-second the toxic value of ionic $\overset{+}{H}$, and may therefore be disregarded in a discussion of the toxic properties of HCl.

Nitric acid, HNO_3; 48, *141*, 384. Ionized in almost the same proportion as HCl (Kohlrausch, '85), HNO_3 proved much more toxic. Inasmuch as the concentration of $\overset{+}{H}$ ions is practically the same as in HCl, and the $\overset{-}{NO_3}$ ion is practically non-toxic, having a toxic value of less than one thirty-second that of ionic $\overset{+}{H}$, we must look for an explanation in the toxic value of the un-ionized molecule, HNO_3. This was found to be approximately 7.7 times that of ionic $\overset{+}{H}$[2]. In other words a molecule of HNO_3 loses nearly seven eighths of its toxic properties for molds on becoming ionized. Krönig and Paul ('97) found that anthrax spores immersed in a $\frac{n}{1}$ solution of HNO_3 for two hours were entirely destroyed. A similar immersion of a similar preparation of spores in the same concentration of HCl permitted the survival of 385 colonies. Preparations of spores immersed in $\frac{n}{16}$ solutions of these acids, however, showed far *less* variation in toxic properties, although the HNO_3 was still distinctly more toxic. This was evidently due to the fact that at the latter concentration the acids were both much more highly ionized.

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[2] See table I, p. 325.

hence both more nearly approximated the toxic value of ionic $\overset{+}{H}$. This toxic power of the un-ionized molecule these workers termed its "specific poisonous effect."

According to this reasoning, then, were HCl and HNO_3 both completely ionized we should expect to find them equally toxic. This matter has been fully tested by Kahlenberg and True ('96). They found that toward Lupinus which is killed by a $\frac{n}{3200}$ concentration of ionic $\overset{+}{H}$, they and all other completely ionized acids with non-toxic anions had *exactly the same toxic value.*

The influence of HNO_3 on germination was much the same as that of HCl, $\frac{n}{1024}$ causing distinct retardation of both germination and early growth. Later, however, a marked difference in the appearance of the cultures manifested itself. Cultures containing the HNO_3 in $\frac{n}{32}$ to $\frac{n}{256}$ concentration produced in nearly every case a much heavier mycelium than in the corresponding cultures in HCl. Fruiting was retarded, but not so greatly retarded as was usually the case where mycelial development was so strongly stimulated.

The stimulation of mycelial development was possibly due to the non-toxic nitrogenous $\bar{N}O_3$ ion. Cultures of Aspergillus and Œdocephalum in $\frac{n}{1}$ solutions of KNO_3, however, did not establish this view, as they did not greatly differ from those in similar concentrations of KCl and K_2SO_4, although the concentration of NO_3 ions would in this case be some forty times as great as in the cultures of HNO_3 showing the most stimulation. A more likely proposition is that it was due to the same factor as the increased toxicity, viz., the un-ionized molecules. The fact that the general appearance of the cultures resembled that of cultures injured by the oxidizing poisons considered later would suggest that power of the nitric acid molecule as being the active influence. The fact that fruiting was not greatly retarded, considering the abnormal mycelial development, is in harmony with this suggestion.

Sulfuric acid, ½ H_2SO_4 ; 61, *205*, 589. As will be observed, $\frac{n}{1}$ of this agent is based on the half molecule in order to make it strictly comparable with the other acids, the others being all monobasic.

H_2SO_4 becomes ionized first into $\overset{+}{H}$ and $\overline{H}SO_4$ ions, but as dilution increases the $\overline{H}SO_4$ ion further breaks up into $\overset{+}{H}$ and $^{-}SO_4^{-}$. The ionization of H_2SO_4 at the average inhibiting point is about 62 per cent. only (Kohlrausch '85). Each 100 molecules then at this concentration breaks up into approximately 124 $\overline{H}$, 76 $\overline{H}SO_4$ and 24 $^{-}SO_4^{-}$ ions. This solution having a greater toxic value than a similar concentration of ionic $\overset{+}{H}$ and the anion being practically non-toxic, the excess of toxic properties must be due to the partially ionized group $\overline{H}SO_4$. By referring to table I it will be seen that the toxic value of this anion is approximately 1.3 in terms of ionic $\overset{+}{H}$.

In this as in the other mineral acids, Sterigmatocystis proved the most resistant, $\frac{n}{2}$ being necessary to kill. Botrytis was the most easily killed, $\frac{n}{16}$ being fatal. Œdocephalum, although requiring $\frac{n}{8}$ concentration to kill the spores, was considerably injured by $\frac{n}{128}$, greatly injured by $\frac{n}{64}$, and produced a very light yeast-like mycelium in $\frac{n}{32}$, which on the third day practically ceased growing. On the whole, H_2SO_4 retarded germination less than HCl and HNO_3.

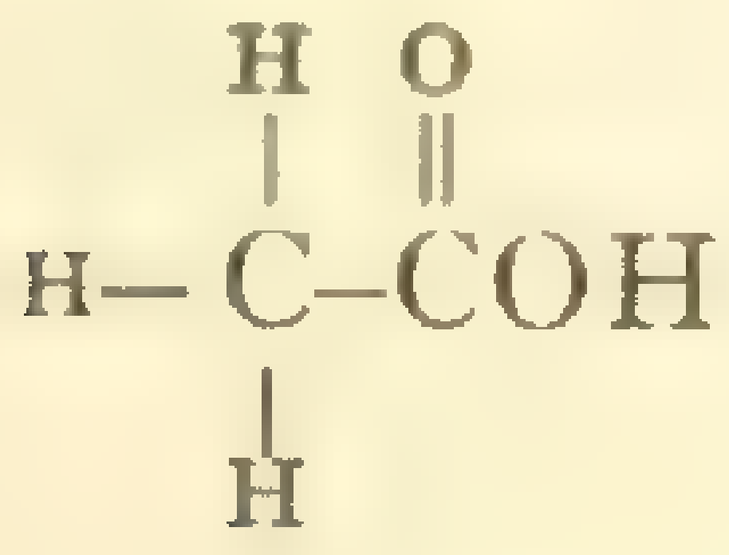
Acetic acid, CH_3COOH ; 25.6, *83*, 314. This acid at the inhibiting point, $\frac{n}{24}$ is but 2 per cent. ionized (Kohlrausch '85). The toxic value of the anion was found to be about $\frac{1}{13}$ $\overset{+}{H}$, but as so small a proportion of the acid is ionized the influence of the anion may be disregarded. The toxic properties of this acid are therefore to be attributed almost wholly to the un-ionized molecule, CH_3COOH, which proves to have a toxic value of 2.8.$\overset{+}{H}$.

Penicillium showed a marked specific resistance to this acid, requiring $\frac{n}{2}$ for 48 hours to kill. This observation was accepted only after repeated trials. Sterigmatocystis, so resistant to the mineral acids, succumbed to $\frac{n}{16}$, and $\frac{n}{32}$ inhibited germination. Botrytis was particularly susceptible, being killed by $\frac{n}{64}$, which is but one eighth the strength of HCl required for the same result. Although so much more fatal to Sterigmatocystis and Botrytis, germination and early mycelial development was much less retarded than with the mineral acids; $\frac{n}{256}$ retarded the germination of Œdocephalum, but in the other forms this concentration had little effect.

That acetic acid should prove so much more toxic to fungi than the mineral acids was not anticipated (Migula '90). Heald ('96) found that it had but one eighth the toxic value of $\overset{+}{H}$ on Zea, and one fourth on Pisum. Kahlenberg and True ('96) found about the same relation with Lupinus. The great variation in protoplasmic resistance to this acid is well shown by the following data: the vinegar eel, *Rhabditis aceti*, thrives in a $\frac{n}{2}$ solution, which is the fatal concentration for Penicillium. Aspergillus spores are killed by $\frac{n}{8}$, those of Sterigmatocystis and Œdocephalum by $\frac{n}{16}$, and those of Botrytis by $\frac{n}{64}$. $\frac{n}{200}$ is fatal to Zea, and $\frac{n}{1600}$ to Pisum.

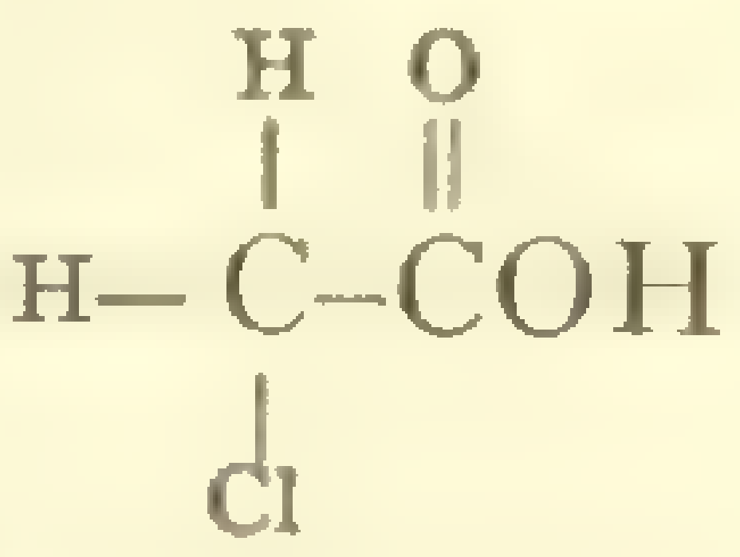
It is of interest to note in this connection that not only are great differences to be expected between different organisms in their resistance to deleterious agents, but different individuals of the same genus and even of the same species may have very different powers of resistance, depending largely no doubt on previous environment. Pfeffer ('95) grew Aspergillus on a nutritive medium containing 8 per cent. dextrose and 1 per cent. acetic acid, and found that the fungus assimilated a far larger amount of acid than the dextrose. It will be noted that $\frac{n}{8}$ (0.7 per cent.) acetic acid proved fatal to the spores of Aspergillus used in this study, and in a 0.17 per cent. solution less than 1 per cent. germinated.

The chloracetic acids.—These acids are formed by the replacement of one, two, and three atoms of H, respectively, in the acetic acid radical by the element Cl; thus—



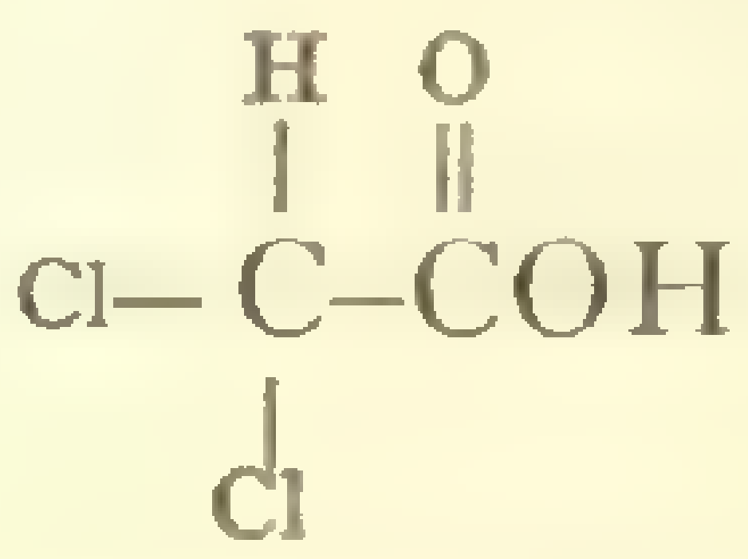
$$\begin{array}{c}\text{H}\;\;\text{O}\\ |\;\;\|\\ \text{H}-\text{C}-\text{COH}\\ |\\ \text{H}\end{array}$$

Acetic acid



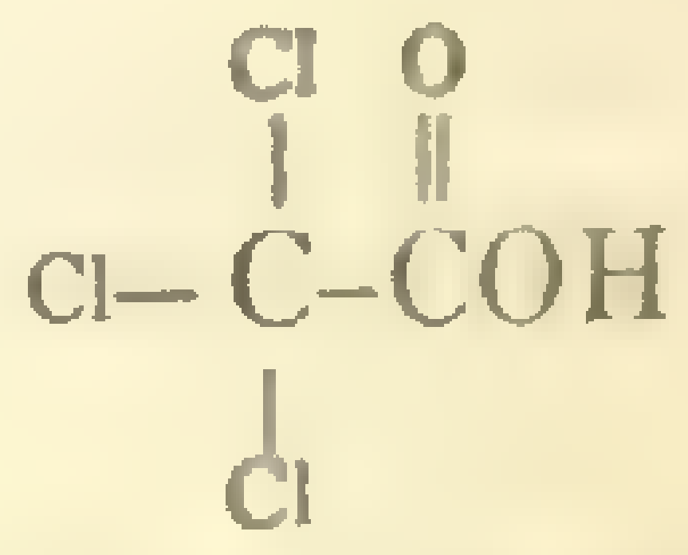
$$\begin{array}{c}\text{H}\;\;\text{O}\\ |\;\;\|\\ \text{H}-\text{C}-\text{COH}\\ |\\ \text{Cl}\end{array}$$

Monochloracetic acid



$$\begin{array}{c}\text{H}\;\;\text{O}\\ |\;\;\|\\ \text{Cl}-\text{C}-\text{COH}\\ |\\ \text{Cl}\end{array}$$

Dichloracetic acid



$$\begin{array}{c}\text{Cl}\;\;\text{O}\\ |\;\;\|\\ \text{Cl}-\text{C}-\text{COH}\\ |\\ \text{Cl}\end{array}$$

Trichloracetic acid

It is a rule that the halogen substitution-products of carbon compounds have a toxic value which bears a close relation to the number of H atoms in the organic radical or hydrocarbon which have been replaced by the halogen. To quote from Davenport ('97): "Beginning with methane, CH_4, we find this substance—marsh gas—innocuous when mingled with air. As the H atoms become replaced by one or more Cl atoms, the poisonous properties increase,—

CH_3Cl is slightly anesthetic,

$CHCl_3$ = chloroform,

CCl_4 is very dangerous, stupefying involuntary muscles."

Many such examples might be quoted, establishing this rule. It was a matter, then, of great surprise to find what, at first sight, seemed to be a direct exception to this rule in the action of the chloracetic acids on the mold fungi. Their critical points were determined as follows:

(Acetic acid	25.6	*83*	314)
Monochloracetic acid	8.8	*58*	64
Dichloracetic acid	10.4	*64*	64
Trichloracetic acid	37	*90*	115

As soon as the experiment was complete the stock solutions used were placed in the hands of a chemist who formed the potassium salt of each by just neutralizing with KOH. These salts are all quite highly ionized, the cathion being ionic $\overset{+}{\text{K}}$ and the anion the acid radicals of the respective acids. The toxic values of these anions, as nearly as could be determined with Aspergillus and Œdocephalum, were as follows:

Monochloracetic - - - -	—CH_2ClCOO,	$\frac{1}{32}$	$\overset{+}{H}$
Dichloracetic - - - -	—$CHCl_2COO$,	$\frac{1}{21}$	$\overset{+}{H}$
Trichloracetic - - - -	—CCl_3COO,	$\frac{1}{11}$	$\overset{+}{H}$

Here the toxic values, although not high, are in the expected order.

Let us next glance at the toxic properties of these acids in a practically completely ionized condition. Kahlenberg and True ('96) found that with Lupinus $\frac{n}{6400}$ monochloracetic permitted growth. The same concentration of dichloracetic was evidently on the line, one plant being killed by it while the check survived. $\frac{n}{6400}$ trichloracetic proved fatal. Ionization being practically complete at this great dilution, we have to deal with ionic $\overset{+}{H}$ and the anions only. The toxic values of such solutions should be equal to that of HCl + the value of the anion. This is exactly what we find. Monochloracetic with its non-toxic anion has the same toxic value as HCl for Lupinus. The others are somewhat more toxic; due doubtless to the influence of the anions, which are apparently relatively more toxic towards the higher plants than towards the molds.

At the concentrations at which they are effective towards the fungi, however, we have an entirely different condition as regards ionization, as may be seen from the following:

Monochloracetic	inhibits at	$\frac{n}{35}$	and is	20	per cent.	ionized.
Dichloracetic	"	$\frac{n}{32}$	"	70	"	"
Trichloracetic	"	$\frac{n}{22.8}$	"	88.8	"	"

This variation in the ionization (Ostwald '89) is the key to the explanation of this apparent deviation from the rule referred to above.

The introduction of Cl into the acetic radical results in two distinct changes in the chemical and physical properties of the acid. (1) A great increase in the toxic properties of the non-ionized molecules. (2) A great increase in the ionization of the acid in aqueous solution of given concentration. That the great ionization often masks the effect of the increase in the toxic properties of the un-ionized part is easily understood when we recall that the toxic value of the ionized portion of an acid is not greater than the toxic value of the ionic $\overset{+}{H}$ + the toxic value of the anion.

Putting all together, we get in brief the following: (1) The replacement of one H in the acetic radical by Cl doubles the toxic properties of the un-ionized portion, and increases the ionization in a $\frac{n}{35}$ solution from 2 per cent. to 20 per cent.; the resultant of these two factors is an increase in the toxic value of about 40 per cent. in a $\frac{n}{35}$ concentration. (2) The replacement of two atoms of H by Cl more than trebles the toxicity of the un-ionized molecule and causes ionization to advance from 2 per cent. to 70 per cent. in a $\frac{n}{32}$ solution. This gives a net increase of about 30 per cent. in toxic properties at this concentration. (3) When all three H atoms are thus replaced by Cl the un-ionized molecule has a toxic value of over five times its original value and ionization advances from 2 per cent. to 88.8 per cent. in a $\frac{n}{22.8}$ solution. At this concentration the effect of the greater ionization is *more* than can be made up by the increased toxic properties of the 11.2 per cent. remaining in molecular form, hence a fall in the toxic properties of this acid at $\frac{n}{22.8}$ concentration as compared with the original acetic.

The values of the un-ionized molecules as worked out in table I, p. 325, show that these acids are very far from being an exception to the rule that the toxic properties of compounds increase with the introduction of Cl into the organic radical. These values are as follows:

Acetic acid,	2.8	times	the value of	ionic $\overset{+}{H}$.
Monochloracetic acid,	4.7	"	"	"
Dichloracetic acid,	9.5	"	"	"
Trichloracetic acid,	14.1	"	"	"

The addition of any highly ionized acid to a solution of a weaker acid throws back the ionization of both, but more particularly that of the one having the low ionization. This method of decreasing the ionization, and thereby increasing the toxic properties, was used with di- and trichloracetic acids. To solutions of these acids HCl was added — molecule for molecule — and the resulting mixture was tested with the five molds. The *calculated* increase of toxic properties due to the *forcing back of the ionization* of dichloracetic acid was found to be 168 units. The experimental test gave an increase of toxicity for the mixture of 147 units more than the *additive* toxic properties of the two acids mixed. In the case of trichloric acid the experimental test gave a slight excess (13 units) over the calculated increase.[3]

Krönig and Paul's ('97) work on anthrax spores is of especial interest here. A preparation of anthrax spores immersed in a $\frac{n}{1}$ solution of trichloracetic acid for 2 hours and afterwards cultured in a favorable medium proved to be entirely sterilized, not a spore surviving. In another test when a similar preparation of spores was immersed in the same concentration for 20 minutes, comparatively few survived. Tests were also made with the same acid at $\frac{n}{16}$ concentration. Spores immersed in this concentration for 56 hours showed much less injury than those immersed for 20 minutes in $\frac{n}{1}$ solution, thus showing clearly that the *efficacy of the acid as a disinfectant was more greatly reduced than could be accounted for by dilution only.* Their work on the other acetic acids, although not extensive, is quite in harmony with the results here recorded for the molds.

Hydrocyanic acid, HCN; 0.36, *3*, 20. This poison, so deadly to the higher vertebrates, has long been known to be much less toxic to less highly organized structures. Extremely fatal to man even in minute quantities, the more lowly organized

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[3] For details of these tests and other data regarding the relation of electrolytic dissociation to the physiological action of acids, see article by the writer in Journal of Physical Chemistry 3:263. May 1899.

Ascaris resists a 3 per cent. solution for 75 minutes. A myriapod (Fontaria) excretes HCN when irritated! The accumulation of many such data has led to the general acceptance of the theory that HCN acts chiefly or wholly upon the aldehydes of the nerve centers (Loew '93). This is no doubt quite satisfactory from the point of view of the animal physiologist, but it leaves us without any explanation for its violent toxic properties toward plants, which have no nerve centers.

Little work seems to have been done with it on plants. Kahlenberg and True ('96) found that toward Lupinus it had double the toxic value of ionic $\overset{+}{\mathrm{H}}$, $\frac{n}{6400}$ proving fatal. To the molds, however, it is relatively a much more powerful agent, having 76.6 times the value of $\overset{+}{\mathrm{H}}$, thus ranking as one of their most fatal poisons. The data on the ionization of this acid are meager. At $\frac{n}{32}$ it gives about one sixteenth the electrical conductivity of acetic acid at the same concentration (Ostwald '85). From this we would judge that the ionization is practically zero at the concentrations with which we have to deal.

The value of the $\overset{-}{\mathrm{C}}\mathrm{N}$ ion was determined by means of the potassium salt, which is quite highly ionized (Kohlrausch '79). It was found to be approximately $8\overset{+}{\mathrm{H}}$ for the molds. Were HCN fully ionized we would expect its solutions to have a toxic value of about $9\overset{+}{\mathrm{H}}$. The fact that the practically un-ionized solutions with which we deal have a value of over *eight times* that calculated for the entirely ionized acid tells for the extremely toxic influence of the un-ionized molecule, HCN.

Aspergillus showed a high specific resistance to this agent, $\frac{n}{32}$ being necessary to kill all the spores. Œdocephalum was particularly sensitive, being injured by $\frac{n}{32768}$, inhibited by $\frac{n}{2048}$, and killed by $\frac{n}{256}$.

In table I the toxic values of the un-ionized molecules of the

TABLE I.

RELATIVE TOXIC PROPERTIES OF UN-IONIZED MOLECULES.

I. As measured by their inhibiting powers.

HCN	$\frac{1}{2}H_2SO_4$	HNO_3	Trich.	Dich.	Monoch.	Acetic	HCl.	Agent	
$\frac{n}{683}$	$\frac{n}{10}$	$\frac{n}{14.5}$	$\frac{n}{22.8}$	$\frac{n}{32}$	$\frac{n}{35}$	$\frac{n}{24}$	$\frac{n}{8.8}$	Concentration inhibiting germination	
7666.	112	163	255	359	396	277	100	Relative toxic value. HCl = 100	
—	62	90.6	88.8	70	20	2		Per cent. ionized at inhibiting point	
—	62	90.6	88.8	70	20	2		Toxic value of cathion in terms of ionic $\overset{+}{H}$	Ionized
—	—	—	8	3.3	.6	—		Toxic value of anion, ditto	
—	62	90.6	96.8	73.3	20 6	2		Total, cathion + anion	
7666	50	72.4	158.2	285.7	375.4	275		Residual units	Un-ionized
100	38	9.4	11.2	30	80	98		Per cent. un-ionized	
76.6	*1.3*	*7.7*	*14.1*	*9.5*	*4.7*	*2.8*		*Value of ionized molecules in terms of ionic* $\overset{+}{H}$	

TABLE I.—(*Continued.*)

RELATIVE TOXIC PROPERTIES OF UN-IONIZED MOLECULES.

II. As measured by their killing powers.

HCN	½H_2SO_4	HNO_3	Trich.	Dich.	Monoch.	Acetic	HCl.	Agent	
$\frac{n}{102}$	$\frac{n}{3.5}$	$\frac{n}{5.3}$	$\frac{n}{18}$	$\frac{n}{32}$	$\frac{n}{32}$	$\frac{n}{6.5}$	$\frac{n}{3.3}$	Concentration killing spores	
3070	104	160	534	960	960	196	100	Relative toxic value. HCl = 100	
—	54.	90	88	70	19.9	1		Per cent. ionized at death-point	
—	54	90	88.	70	19.9	1		Toxic value of cathion in H	Ionized
—	—	—	8	3.3	.5	—		Toxic value of anion in H	
—	54	90	96.	73.3	20.4	1		Total cathion + anion	
3070	50	70	438	887	940	195		Residual units	Un-ionized
100	46	10	12	30	80.1	99		Per cent. un-ionized	
30.7	*1.1*	*7*	*36.5*	*29.6*	*11.7*	*1.97*		*Value of un-ionized molecules in terms of ionic* $\overset{+}{H}$	

different acids, as shown by their inhibiting and killing powers toward the spores of the five molds, are approximated. Too much importance must not be attached to the *exact numerical* value here expressed for the different molecules, for several reasons. We do not know the reaction of the acids toward the nutrient medium in which we grow our plants; we do not know the exact effect of the salts and sugars present on the ionization of the acids; and were it possible to eliminate every factor causing doubt or error, we should undoubtedly find the relative toxic properties of the molecules varying with almost every plant tested. Let me repeat: the exact numerical values here given are *not* significant. The order and general proportions of these values *are* significant. The emphasis is laid on part I of this table for reasons already given.

Line 1 gives the strength of the various acids required on an average to inhibit germination. Line 2 is developed from line 1. It gives the relative toxic properties of the acids expressed numerically, HCl being taken as 100 for a basis of comparison. The ionized and un-ionized portions are considered separately, lines 4, 5, and 6 being devoted to the former, and 7, 8, and 9 to the latter. Line 4 gives the toxic value of the cathion in units of ionic $\overset{+}{H}$; line 5 that of the anion. Line 6, being the total of 4 and 5, gives that portion of the total toxic value of the acid which is to be attributed to ionized portion. Line 7 gives the "residual units." In other words, that part of the total toxic value to be accounted for by the un-ionized portion. Line 8 gives the percentage of such un-ionized molecules present at the inhibiting point. Line 9, the quotient of the residual units divided by the percentage of un-ionized molecules, gives in terms of ionic $\overset{+}{H}$ the toxic value of the different acid molecules.

Part II is worked out similarly, and has reference to the toxicity of the acids towards the molds, as shown by their power to *kill* the spores.

[*To be concluded.*]

THE DEVELOPMENT OF THE MICROSPORANGIUM AND MICROSPORES IN CONVALLARIA AND POTAMOGETON.

KARL M. WIEGAND.

(WITH PLATES XXIV–XXV)

DURING the past decade perhaps no one portion of the field of botany has been worked upon so much as that which deals directly with the organs concerned in the sexual process. Especially is this true of the higher plants, but a careful survey of the present condition of our knowledge on this very point shows that some of the most vital questions have as yet received no solution. The researches of such men as Hofmeister, Strasburger, Guignard, and Warming have discovered facts in regard to the ovule, the embryo-sac, and the cell which have already become so universally known as to need no further mention here. But we still know almost nothing about the essential significance of some phenomena of most common occurrence, and many questions have as yet been investigated only in connection with so few plants that generalizations are extremely unsafe. It was principally with the hope of increasing, if only by a few species, the range of observations that the present studies were undertaken.

The choice of subjects signifies very little. It was influenced much more by the necessity of using plants obtainable at certain times rather than by an idea that they all represented different types of structure.

METHODS.

The methods employed in this work differ in no essential way from those so often described in recent cytological works, consequently it is scarcely necessary to repeat them again in detail. The following general statements are intended rather for those who wish simply to know the stains, fixing agents, etc., chosen for the work.

The Flemming chrom-osmo-acetic acid solution has of late years proved of such value that it must now be regarded as the very best fixing agent for cytological work. For the following studies material from no other fixing agent was used, although some was put up in alcohol, sublimate, and picric acid. None of the latter gave satisfactory results. The secret, if there is any, in the use of the Flemming solution seems to lie in obtaining rapid penetration. To accomplish this the material cannot be subdivided too much, and when possible even the anthers themselves should be cut open. From six to twelve hours is sufficient for complete fixation. The black discoloration caused by the osmic acid was removed by the aid of hydrogen peroxid either upon the sections themselves or preferably upon the material *in toto*.

For clearing, cedar-wood oil gave the best results. It was always added with great care in order to avoid too rapid change of density, otherwise collapse of the cells often occurred. The paraffin used possessed a melting point of 54°. This also was added with the same degree of care. The sections ranged in thickness from four to six and two thirds microns, and were all cut on a Minot-Zimmerman revolving microtome.

Considerable experimentation was necessary before a suitable staining method could be devised. Among others, Rosens' fuchsin-methylene blue method,[1] iron-hæmatoxylin and the Flemming safranin-gentian-violet-orange combination were the most important. The latter was at length almost exclusively employed, and was found very satisfactory. As a general nuclear stain, however, better results were obtained by the omission of the safranin, and at the same time a large amount of time was saved. The orange G was always used in a very dilute solution, and for a very short time, from 15 sec. to 2 min. giving the best results. For a chromosome stain the gentian-violet was allowed to act for a short time only, but for differentiating the spindle fibers and kinoplasmic radiations, much better results were obtained with a very weak solution (2–3 drops of the stock solution made according to Lee in 100^{cc} of water) acting for from 12 to 24 hours. After washing out in absolute alcohol, further differentiation was obtained with clove-oil, or in the case of Potamogeton, preferably with anilin oil. The action was then stopped with bergamot oil before mounting in balsam.

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[1] Beiträge zur Kenntniss der Pflanzenzellen. Cohn's Beiträge 5:443. 1892.

CONVALLARIA MAJALIS L.

The Liliaceæ have so far furnished some of the very best subjects for cytological study. To those already studied must now be added another good type, namely Convallaria. This plant has unfortunately up to the present time received very little attention, although the large nuclei and long rod-like chromosomes almost equal Lilium in the ease with which they may be studied. The published observations are at present limited to those of Strasburger on the pollen of *C. Polygonatum*, and on the endosperm of *C. majalis*.[3] The observations on both of these are very brief.

The material for this study was obtained from plants grown in the University greenhouses. They were here under a constant though moderate condition of forcing, which thus enabled one to obtain an unusually large proportion of dividing nuclei. The progressive development of the flowers in the raceme makes it possible to find many stages on a plant all at one time. To insure rapid penetration of the fixing agent the upper and lower ends of each bud were cut away, thus exposing directly the cells of the anther.

In order to determine if there might not be a relation between the nuclear division and the environment of the plant, especially as to the amount of light, humidity, etc., several experiments were made. In the case of Convallaria only the effect of light could be studied, since the other conditions in the greenhouse were practically the same. In order to test the effect of light, material was collected at various times during the day and night. The results, however, were wholly negative. Spindles were found in about the same proportion in every collection.

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[2] Befruchtungsvorgänge bei den Phanerogamen 171. 1884.

[3] Theilungsvorgänge der Zellkerne 43. 1882.

THE DEVELOPMENT OF THE MICROSPORANGIUM.

In 1873 Warming's important work on the development of the anther apeared.[4] In this paper we find for the first time a correct description of the succession of cell divisions resulting in the formation of the archesporium and the anther wall. It was not followed up, however, by other investigators, and even in late years nothing of importance has been done along this line. The most important of the subsequent papers is undoubtedly that of Engler in which Orchis is taken up in detail.

Warming established the fact that the archesporium arises from the daughter cells resulting from the division of the hypodermal layer at each corner of the anther. If the hypodermal cells form a true layer, then the archesporium will usually also be in the form of a layer; but in some cases the hypodermal cells may be reduced to one, and the resulting archesporium in that case is simply a vertical row of cells. In any event, there is almost no subsequent division in the archesporium, growth being confined entirely to an increase in size of the existing cells. Of the two original daughter cells of any hypodermal cell, the inner gives rise to the archesporium, the outer to the anther wall. By two or three periclinal divisions progressing in a centrifugal manner a radial row of cells is formed, the inner cell of which becomes the tapetum, the outer the endothecial layer, and the rest finally disintegrate. Although many of the divisions are periclinal, some are radial and others transverse, by which means accommodation is provided for the growth of the archesporium. He finds also that cell division in the epidermis is almost entirely radial and transverse, and this structure remains always one-cell thick. His subjects for investigation, however, included only one monocotyledon; and Engler,[5] who attempted to show more clearly the application of Warming's laws to the monocotyledons, used Orchis as the only type. At present, therefore, our knowledge of the details in this group is very meager.[6]

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[4] Untersuchungen über pollenbildende Phyllome und Caulome. Hanstein's Bot. 2: 1. 1873.

[5] Beiträge zur Kenntniss der Antherenbildung. Pringsh. Jahrb. f. wiss. Bot. **10**: 1876.

In Convallaria the early stages in the development of the anther are not so easily understood as are those of the dicotyledons; but after the investigation of a large number of preparations it seems probable that the following is the proper interpretation, not alone for Convallaria but also for many other monocotyledons. The earliest stages obtained show in cross-section a four-angled anther with a radial row of cells at each angle, which apparently result from the division of a single hypodermal cell.

The condition at this stage is represented in *fig. 1*. The innermost cell, the primary archesporial cell, very soon divides in various directions until a considerable mass of tissue is formed. This division takes place very early, so that the final number of archesporial cells is formed even before the anther has become obviously lobed. At this stage it is only with considerable difficulty that the archesporium can be distinguished from the wall (*fig. 2*). The original radial row of cells, descendants of the primary hypodermal cell, may often be recognized for a considerable time after they are first formed, but in some cases one or two cells on either side may also divide several times in a radial direction. The greater portion of the wall, however, is derived from a few irregular divisions of the cells at each side of the archesporium, while the epidermis is at the same time increased by a few anticlinal divisions. In addition to this the cells between the archesporium and the connective may also undergo a few radial divisions. As a result the wall-cells in the older anthers do not stand in distinct radial rows as in the dicotyledons, simply because they were not all derived by radial division; but, notwithstanding this, there are quite regularly three or four layers of cells differentiated entirely around the sporogenous tissue. These stages were found in the autumn previous to the time of flowering, and all subsequent growth, both in the archesporium and in the wall, is due entirely to the increase in diameter of the cells already formed. This was determined by an actual count of the cells in a great many cases.

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[6] While in press the following papers have appeared:

GUIGNARD, L.— Le développement du pollen et la réduction dans le *Naias major*. Arch. d'anat. Microscop. **2**: 455. 1899.

CALDWELL, O.— Life history of *Lemna minor*. BOT. GAZ. **27**: 37. 1899.

FULLMER, E. L.— The development of the microsporangia and microspores of *Hemerocallis fulva*. BOT. GAZ. **28**: 81. 1899.

In most dicotyledonous anthers the archesporium becomes distinct at a very early period. No such sharp demarcation exists, however, in the monocotyledons. In all of the cases studied by the writer, and in those treated by Warming and Engler, the transition from the wall to the archesporium is so gradual, especially in the younger conditions, that only the most careful study enables one to distinguish accurately between them. Although it is difficult to distinguish them solely by their form, it is not so difficult when their cytoplasm is considered. In Convallaria the cytoplasm of the archesporial cells becomes so modified that by its structure alone one can recognize the future mother-cells. The change consists in an increase in the abundance of the cytoplasm, as well as in the formation of a much finer network with scarcely any indication of a granular structure. Taking this into consideration, the wall is now readily seen to consist of four layers.

From the above description it will be seen that there really is here a special case of Warming's law as formulated for the dicotyledons, and not an entirely new process. In other words, the archesporium seems to arise entirely from the division of one or two hypodermal cells rather than from a layer of such cells, and the wall is formed mostly from cells adjacent to the archesporium.

At a slightly later period the fourth or innermost layer of the wall begins to enlarge. The cells grow considerably, and soon might be mistaken for archesporial cells if it was not for the difference in the cytoplasm. This layer is the tapetum (*fig. 3*).

The history of the tapetal nucleus has been studied by

Strasburger in Malva, but not in detail.[7] In this plant two nuclei were found in each cell. Guignard also figured two nuclei in the tapetal cells of Lilium, and it now seems probable that the phenomenon is quite general, as Strasburger in the above cited work states. It was originally believed that these two nuclei were formed by direct division; but Strasburger[8] has shown that this was really a process of fusion, and that the two nuclei were formed at an earlier date by the ordinary indirect method.

In Convallaria, at the period just preceding synapsis, the already enlarged tapetal cells contain only one nucleus; but during synapsis, and even up to the first pollen-mother-cell division, the nuclei one by one divide by the mitotic method. Such spindles are seen in *fig. 4*. It is probable that every tapetal nucleus finally undergoes division, but this could not be accurately determined because of the subsequent fusion. The different stages in the fusion of these nuclei were sufficiently frequent after the first division of the pollen-mother cell (*fig. 5*). Cases could often be found even in anthers where tapetal spindles also occurred. It is doubtful if all the pairs of nuclei fuse — in fact, it is probable that they do not, since many remain distinct even after the tapetum shows signs of disintegration.

The wall of the mature anther in Convallaria presents no new features. It is composed of a conspicuous epidermis, a well-developed fibrous layer (endothecium) with beautiful spiral markings, and the remains of the other wall layer (*fig. 6*). No remnant of the tapetum is now left. The time of disappearance of this structure was interesting because it occurred in some cases as early as the pollen-mother-cell stage, while in others not until after the pollen grains were mature. The time is seldom the same even in anthers from the same bud.

THE NUCLEUS OF THE ARCHESPORIUM.

The very earliest stages of the archesporial nucleus in Convallaria differ only slightly in appearance from true vegetative nuclei (*fig. 7*). The linin thread is exceedingly thin and fine as well as extensive. Careful examination shows that it is not a continuous ribbon at this period, but rather an anastomosing network in which large granules of chromatin of unequal sizes are imbedded, giving it thus a more or less knotty appearance.

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[7] Ueber den Bau und das Wachsthum der Zellhäute 89. 1882.

[8] Theilungsvorgänge der Zellkerne 99. 1882.

At a period quite early in the development of the anther the chromatin network contracts into a dense and ultimately spherical mass, which in most cases is in contact with the nuclear membrane, but may also lie free in the nuclear cavity (*fig. 8*). The ball becomes so dense that it is ordinarily impossible, even with the weakest stains, to distinguish the separate threads of which it is composed, except at the periphery where they project into the nuclear cavity outside. This condition is synapsis in Convallaria. The nucleolus does not lie inside the mass and adjacent to the membrane, as has been claimed for some other plants. On the contrary, it always lies outside so far as could be determined, but usually in contact with the mass and at the side away from the wall more often than toward it.

Judging from the number of preparations obtained, the nucleus must remain in the synapsis condition for a considerable time. The first indication of the return from synapsis is found in the gradual separation of the outermost linin threads, and finally the whole mass becomes more open (*fig. 9*). But a very strange thing now occurs. At the moment when the chromatic thread is spreading out, between its meshes and in the cavity outside are to be seen large granules, or more properly speaking masses of various sizes which are decidedly chromatic, in fact stain exactly like chromatin. The nucleolus meanwhile still remains intact. Where these bodies come from or where they go could not be determined. They were always present, however, at this stage. The masses were often as large as the nucleolus but more often smaller. It might be suggested that in some way the nucleolus becomes fragmented either naturally or by the action of the reagents, but the preparations do not support this view. In very weakly stained nuclei the nearly colorless nucleolus with its central vacuole could be plainly seen, while in the same nucleus the expelled masses were stained dark like the chromosomes at the time of division. Again it is possible that the chromatin had left the linin thread and collected in the above manner before the material was fixed. Or perhaps the reagents caused such a change in the chromatin. The investigation of fresh material alone can decide whether the process is normal or artificial.

A remarkable change has also taken place in the chromatin thread. Before synapsis it was a network containing very large irregular granules, now it is a spirem with the granules much reduced in size and more uniform. For the most part they are but slightly broader than the scarcely thickened linin. These above mentioned peculiarities were found not in one case alone, but in a great many preparations, in fact in every preparation that contained the right stage.

Synapsis has now been found in many plants, in all of which it always seems to be a natural condition, and it is quite probable that it will prove to be a universal phenomenon, occurring at a certain period previous to each heterotypic division. Besides this, it has now been shown to occur in at least a few animals. Among plants it has been found in the Liliaceæ by Strasburger, Sargant, and others; in the Hepaticae by Farmer; and in Potamogeton and Acorus by the writer, in addition to several other plants, the studies on which have not yet been published. There now seems to be little doubt that the condition is a natural one for the following reasons. It may be found in the fresh material, at least in Convallaria and Lilium. Moreover, it always occurs at the same stage in the development of the anther. Various structural changes in the chromatin thread always accompany it. In addition to this, many preparations show that the mass may be deposited at any side of the nuclear cavity without reference to its position in the anther.

Considerable uncertainty has always existed as to just what synapsis really is. The term was introduced by Moore[9] in the year 1895, but the description was so brief that it is somewhat difficult to understand just what the author had in mind. It seems probable, however, that the condition found by him was the same as that described above for Convallaria. He was able to demonstrate the appearance of the same phenomenon also in animals, especially in Triton, thus emphasizing the fact that the steps in the maturation of the sexual gametes is to a certain extent similar in both plants and animals.

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[9] Essential similarity of the chromosome reduction in animals and plants. Annals of Botany **9**: 435. 1895.

During the past few years synapsis has been described by several authors, most of whom now consider it to be a natural condition of the cell, although it seems probable that the phenomenon described is not the same in all cases.

Farmer[10] found a contracted condition of the chromatin thread in the spore-mother-cells of the Hepaticae, but the figures do not show as great a contraction as is found in Convallaria. He states that the nuclei at this stage are difficult to fix, often showing signs of fragmentation, and that there is usually a chromatic change in the cell.

Convallaria has so far shown no case similar to that described by Miss Sargant[11] for Lilium, in which two rows of dots were found on the thread before synapsis. A double row in the former plant is found only very late in the history of the spirem; neither does the nuclear membrane disappear or even become indistinct during synapsis. Otherwise the description and figures are quite similar to Convallaria. In some preparations an extrusion of granules from the contracted mass was found, but was interpreted as a fragmentation of the nucleolus rather than as a separation of portions of the chromatin.

The conflicting results obtained are probably due, in part at least, to the fact lately emphasized by Strasburger,[12] that several distinct phenomena have been referred to synapsis. Only one of these is a normal condition, and to this the term synapsis properly belongs. The others are probably caused by the action of the reagents.

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[10] On spore formation and nuclear division in the Hepaticae. Annals of Botany 9:482. 1895.

[11] The formation of the sexual nuclei in *Lilium Martagon*. Annals of Botany 10:457. 1896.

[12] Karyokinetische Probleme. Pringsh. Jahrb. f. wiss. bot. **28**: 158. 1895.

Mottier's[13] figures representing synapsis in Lilium do not show nearly as much contraction as in Convallaria. From the description it would seem also that they represent a much later stage in the development of the spirem than the one in which synapsis occurs, either in Convallaria or in Potamageton.

FIRST NUCLEAR DIVISION OF THE MOTHER-CELL.

The spirem stage preceding division is very well marked in Convallaria (*fig. 10*). The much coiled wire-like thread found immediately after synapsis gradually increases in thickness, and the chromatin granules become less prominent. One large nucleolus and usually two or three smaller ones are present at this period. The longitudinal splitting of the chromatin thread is accomplished so quickly that it was found impossible to observe the successive steps in the process. At the same time the thread becomes considerably thicker than immediately before the division (*fig. 11*). It is difficult to understand just how this doubling takes place, because at the very first indication of such a condition the threads are already separated. They lie parallel with each other and slightly coiled. The further development indicated a more or less complete subsequent fusion of the parts, so that before passing into the nuclear-plate stage the dual nature is entirely lost except for an occasional lobing at either end (*fig. 12*). Not even the granules are longer visible, and the chromosomes are at this stage apparently homogeneous. This is probably only apparent, however, and due really to the density of the stain and the close proximity of the parts.

After the chromatin thread has become double, besides being thicker than before, it also possesses fewer coils, which is probably due to a longitudinal contraction of the whole spirem. At about the time when the nuclear membrane disappears the thickened chromatin band segments into the individual chromosomes

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[13] Beiträge zur Kenntniss der Kerntheilung in den Pollenmutterzellen einiger Dikotylen und Monokotylen. Pringsh. Jahrb. f. wiss. bot. **30**: 175. 1897.

(*fig. 12*). The successive stages in the process of segmentation could be easily traced. The constrictions gradually become deeper and deeper, while at the same time the chromatin is withdrawn from the constricted regions. The resulting segments were always of the same length as the mature chromosomes appearing on the nuclear plate. Repeated examination of these stages failed to reveal a V-shaped bending back of the segments corresponding to that described by many investigators for Lilium. Moreover, the chromosomes do not have four lobes at the end, as would be more likely the case if they were formed by the folding-back process, but only two. It seems, therefore, that in Convallaria at least the chromosomes, although double in nature and hence sometimes showing a longitudinal split, are always straight and formed simply by the transverse fission of the chromatin thread.

The changes which the chromosomes pass through while on the nuclear plate are very difficult to make out, and consequently little that is definite can be said about them. The segments seem to be straight or slightly curved and lie mostly in a radial manner on the plate (*figs. 14, 19, 20*). The earlier stages show a nearly cylindrical chromosome, but very soon this becomes changed into the characteristic +-like structures which are very commonly seen at this stage. These structures seem to be formed, as Belajeff[14] and Strasburger[15] have shown, by a simultaneous splitting at each end of the cylinder, but in perpendicular planes. The inner forks are drawn apart by the spindle fibers, while the two outer ones separate in the plane of the nuclear plate. In many cases a fissure may be seen to extend from the apex of one long arm directly through the middle of the chromosome (*fig. 20*).

The two V-shaped segments resulting from the separation of the two halves of the + pass to the poles in the ordinary manner (*figs. 14, 15, 22*). The daughter segments proceed with the

[14] Zur Kenntniss der Karyokinese bei den Pflanzen. Flora, Erganzungsb. **79**: 434. 1894.

[15] Karyokinetische Probleme. Pringsh. Jahrb. f. wiss. Bot. **28**: 183. 1895.

angle of the V in front, as one might expect if the spindle fibers are assumed to be exerting a pull upon them. They are always more slender than the parent chromosomes, which would be the case if a division of the original substance had taken place. Occasionally a V will straighten out and lie along the spindle fiber, reaching almost from the equator to the pole.

The spindle in Convallaria is formed in the same way as in Lilium. The disappearance of the membrane is exactly coincident with the appearance of the kinoplasmic threads which immediately penetrate the nuclear cavity, and also extend outward into the cytoplasm (*fig. 12*). Very little light, however, could be thrown on the fate of the nucleolus, and its disappearance was very sudden. The multipolar spindle is not very distinct in this plant (*fig. 13*), and the poles scarcely ever extend much beyond the limits of the old nuclear membrane, and are often difficult to distinguish at all. The bipolar spindle is usually truncate at the poles, but unlike that of Potamogeton, it is broad and barrel-shaped, possibly due to the large number of chromosomes (*fig. 14*). In this respect it is similar to Lilium. In no case was there even so much as a granule present at the pole, or any thing that could be mistaken for a centrosphere. A strong nuclear plate follows the division, resulting in a cross wall separating the cell into two hemispherical parts.

SECOND NUCLEAR DIVISION OF THE MOTHER-CELL.

The resting stage between the first and second divisions in Convallaria is very short. One may often find the nuclei at one end of an anther in the cell-plate stage, while those at the other end have formed the nuclear plate for the second division. The daughter nuclei do not seem to pass entirely into a normal resting condition. So far as could be determined, no nucleolus ever appears, nor does the nuclear membrane become well developed, however a very delicate membrane may often be observed. The chromosomes apparently retain their identity throughout this stage. From the pole view it can be determined that they still possess their V-shaped form (*fig.* 16).

The transition from the resting stage to the second spindle is very abrupt. Two poles are formed in the cytoplasm, which move farther apart, so that a bipolar spindle is very quickly formed (*fig. 17*). In the preparations examined, no polar radiations or multipolar spindles were found. The already distinct chromosomes merely move together toward the center in order to form the nuclear plate. Even now most of them retain the V form, and only an occasional one becomes nearly straight. They are very irregularly arranged, so that the long arms of some project outward toward the poles, giving a ragged appearance to the plate, thus distinguishing it immediately from the plate formed in the heterotypic division.

It was found impossible to determine absolutely the nature of the segmentation, but from a study of all the stages obtainable it seems probable that the V is divided transversely. This of course would not mean a transverse division of the original chromosome, if the processes described for the first division are the true ones, but rather the completion of a second longitudinal splitting. No figures were found in any of the spindles that could be interpreted as a case of undoubted longitudinal splitting of the V after coming on to the nuclear plate of the second spindle. On the other hand, the nearly straight segments moving along the spindle toward the poles are all much shorter than the Vs, but of approximately the same diameter instead of narrower, as one would expect if longitudinal splitting had taken place.

The later stages are all perfectly normal. The chromosomes arrange themselves in the daughter nuclei and appear at length to fuse into a continuous chromatin thread. The nuclear membrane does not appear at once, but by the time the young pollen grains are differentiated it is usually evident.

The spindle during the second division, both before and after the passage of the segments to the poles, is much less distinct than during the first division, and it is composed of fewer fibers. After the daughter nuclei are formed, a cell-plate is deposited in the usual manner. The spindle now disappears and the tetrad division of the pollen-mother-cell nucleus is complete.

The number of chromosomes in Convallaria is quite large. A count in the nuclear plate stage showed eighteen segments as the reduced number. The same number may be counted during the subsequent resting stage and also after the second division.

THE MICROSPORES.

After the second division of the mother-cell nucleus the young pollen grains do not separate immediately, but remain a short time inclosed in the thickened walls of the parent cells. With little difficulty one can follow all the steps in the process of dissolution which these walls undergo. First the increasing sponginess of the already thick wall; a simultaneous differentiation of its inner layer destined to become the wall of the spore; and finally the complete solution of the outer part, leaving the young pollen grains united only by the intervening walls. These apparently split at once into two layers, thus freeing the members of the tetrad.

The pollen grains at first are quite small, and possess thin purple-staining walls, finely granular cytoplasm similar in consistency to that of the somatic cells, and a highly chromatic nucleus which occupies about one fourth of the cell-cavity (*fig. 23*). The further changes are mostly normal. The pollen grains, which from the first are elliptical, gradually increase in size until their volume is more than doubled. The wall increases in thickness, and the whole grain assumes a bluer tinge with gentian-violet.

A short time before the flower opens, the nucleus undergoes division, whereby a generative cell is cut off (*fig. 24*). This cell is lenticular in form, and separated from the general cavity of the grain by a distinct cell wall. The generative nucleus is exceedingly chromatic, so much so in fact that it stains almost a homogeneous dark purple with the gentian-violet. The generative cell in Convallaria seems to differ from those in most of the monocotyledons described by other writers in not separating at an early period from the wall of the pollen-grain. It apparently remains in all cases attached until the time of pollination.

The division of the generative nucleus into the two sperm nuclei must take place in the pollen tube, good stages of which were not obtained. Except in the one point above mentioned, the microspores of Convallaria do not differ in any essential way from those described by Strasburger[16] for a large number of the higher monocotyledons.

POTAMOGETON FOLIOSUS RAF.

The numerous investigations recently made upon plants belonging to the orders Alismaceæ and Naidaceæ have shown that many peculiar conditions are to be found among these groups of monocotyledons. During the past summer the writer was able to procure excellent material of *Potamogeton foliosus* in the ponds about Ithaca; and it was decided to make a detailed study of this plant for comparison with the studies already made by others. No one seems to have investigated this genus from a cytological standpoint.

Of the papers on nearly related plants must be mentioned that on Naias by Magnus,[17] on Naias and Zannichellia by Campbell,[18] and on Alisma[19] and Sagittaria[20] by Schaffner.

The material was collected during the months of July and August, at which time the oldest flowers are just producing fruit. The floral spikes mature in succession as the plant branches, so that the very youngest flowers and also the fruits may be found upon the same individual. There being no cutinized layer surrounding the bud, the latter is especially easy to penetrate with the fixing agent. The material used for this study was therefore in exceptionally good condition. As in the case of Convallaria, the collections were made at certain times during the day and night, and the result also was exactly the same.

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[16] Befruchtungsvorgänge bei den Phanerogamen 22. 1884.

[17] Beiträge zur Kenntniss der Gattung Naias. Berlin. 1870.

[18] A morphological study of Naias and Zannichellia. Proc. Calif. Acad. Sci. III. 1:1. 1897.

[19] The embryo sac of *Alisma plantago*. BOT. GAZ. 21:123. 1896.

[20] A contribution to the life history of *Sagittaria variabilis*. BOT. GAZ. 23:252. 1897.

THE DEVELOPMENT OF THE MICROSPORANGIUM.

To trace the development of the anther, and especially the differentiation of the microsporangial archesporium in Potamogeton, is no less difficult than in Convallaria. Oddly enough, the only monocotyledonous type studied by Warming was one of the Naiadaceæ, namely Zannichellia. Warming thought that in this plant the process was essentially identical with that in the dicotyledons, but these results may be questioned, owing to the apparent paucity of material at his command.

The present study of Potamogeton seems to throw a little more light on the problem. In the young anther, which at maturity is always two-celled, there is found at each of the two more prominent angles of the quadrangular cross-section a single hypodermal cell, which at this stage is slightly larger than the surrounding cells and richer in protoplasm. This presently is divided by periclinal walls into two, and later into three daughter cells, each produced probably in centrifugal succession (*fig. 25*). The innermost of this series now immediately begins to enlarge, and becomes at once the primary archesporial cell. This cell undergoes rapid division, resulting at length in a number of cells, all formed from this one original archesporial cell. The irregularity in arrangement, and the gradual decrease in size from the center toward all sides, nevertheless suggest that some may owe their origin to the division of the surrounding tissue. The same gradual decrease in size takes place also on the side of the archesporium toward the connective.

It will be seen from the above account that the tapetum here and in Convallaria is not a morphologically distinct structure until at a comparatively late period in the development of the anther. It is not until the stamen is half mature that the archesporium becomes distinct from the wall. It can always be recognized at this period by the finely granular contents of the cells, just as was the case in Convallaria. No tapetum can be distinguished for some time. The cells of the inner layer of the wall, which from the first are smaller than the central cells, gradually take on a dense and partially disorganized appearance. There is no longer any doubt but that the tapetum is differentiated from the wall, rather than from the archesporium, as a hasty inspection would seem to indicate.

The wall of the anther at this stage is composed of three, or rarely four, layers of cells (*fig. 26*). The outermost of these layers remains almost unchanged until the anther is mature, and is indeed the true epidermis. The other two or three layers have some of their cells arranged in more or less distinct radial rows, suggesting, as in the first case, that each row is the derivative of one hypodermal cell. The greater portion of the wall, however, is formed, as in Convallaria, from the cells of either side of the archesporium, and these are not necessarily derivatives of a hypodermal cell. Indeed, so far as could be determined, the growth was brought about exactly as in Convallaria. During the maturation of the anther the behavior of the cells is normal. The third layer undergoes disintegration, as does also the fourth, which is the tapetum. The epidermis remains normal, while at the same time the second layer becomes thicker walled than the rest, acquires spiral or reticulated thickenings, and is indeed a true endothecial layer (*fig. 27*).

Campbell considers the anther of Naias to be a so-called "caulome" structure, in which are early differentiated plerome and periblem, the upper cell of the plerome cylinder becoming the archesporium. This in itself does not preclude a process similar to that described above for Potamogeton; although Campbell himself is quite certain that the origin of the archesporium in Naias is not traceable to a single cell. He found the wall composed of only two layers besides the epidermis, instead of three. In Zannichellia the same difficulty was found in tracing the development, but this was probably because the tapetum was not counted as a wall layer. The archesporial cells are here also at first scarcely distinguishable from the adjacent cells. In this plant there are three layers surrounding the archesporium, all of which finally become disintegrated.

The complete disintegration of the tapetal cells in Potamogeton is almost coincident with the divisions of the pollen-mother-cell.

When the young pollen grains are free in the anther, therefore, only a disorganized mass of protoplasm is in the position formerly occupied by the tapetum. This substance is very soon distributed among the pollen grains, where it possibly serves as nutriment. The tapetal cells of Potamogeton never contain two nuclei. In this respect, therefore, they differ decidedly from Convallaria.

THE ARCHESPORIUM AND MOTHER-CELLS.

Division in the primitive archesporium ceases at an early period, after which the development is confined to growth and constitutional changes in the cells already formed. The definitive archesporial cells are at first quite small, but during the long period of growth that now commences they double or even triple their original size. The mature pollen-mother-cell contains a very large nucleus surrounded by abundant cytoplasm. Unlike most monocotyledons, the cell wall here remains very thin, and does not become irregularly thickened, as in Convallaria and Lilium (*fig. 35*). A similar condition has been observed also in Naias and Zannichellia. A very short time, therefore, is required for disintegration, which undoubtedly accounts for the almost immediate separation of the young pollen grains.

THE ARCHESPORIAL NUCLEUS.

Potamogeton belongs to an entirely different class from Convallaria so far as the nuclei are concerned. The Lilium type, to which the latter plant belongs, possesses the well-known dense spirem and the large oblong chromosomes. The nuclei of Potamogeton are apparently very poor in chromatin. The few chromosomes are small and spherical and the spirem very meager. A detailed comparison with Convallaria, therefore, will be especially interesting.

The very young archesporial nuclei in Potamogeton are scarcely different from the surrounding vegetative nuclei. They are surrounded by a definite membrane, have a large nucleolar-like body, and a very poor linin network, which lies close to the wall (*fig. 28*). As the cell gradually expands, the nucleus also increases in size. The nucleus just before synapsis has already acquired nearly its full size, and the linin network composed of very slender threads is plainly visible. In it are irregularly distributed a few large and small granules of chromatin. Both the linin and the granules are exceedingly meager as compared with Convallaria. The nucleolus is a gigantic body, much larger than those in most plants, and takes the gentian-violet stain readily, making it thus a very striking object in the cell. Attached to it on one side is a small wart-like body only slightly larger than the largest chromatin granules. Rarely two of these are present. This body appears very much like a bud produced by the nucleolus itself, but in reality is not. If specimens are examined which have been poorly stained, in which the gentian-violet has been mostly washed out, this little body remains dark much longer than the nucleolus. A fine double stain can often be thus obtained. The nucleolus takes the orange in that case, while the other body stains violet. From the fact that this larger body always stains like ordinary chromatin, and since there is scarcely any chromatin upon the linin thread, the writer is inclined to believe that it is not the nucleolus, but a large mass of chromatin similar to those found in many animal cells. The nucleolus is possibly the wart-like body attached at its side. The behavior of the larger body when the chromosomes are being formed seems also to support this view.

The synapsis stage in Potamogeton is even more marked than in Convallaria (*fig. 29*). The linin network contracts into a globular mass lying in contact with the nuclear wall, but not noticeably pressed against it. With the highest magnification, the central part of the mass still appears too dense to distinguish any structural characters. On the periphery, however, the free ends of the network may be seen easily. The large nucleolus, accompanied by the wart-like body, remains in its central position in the nucleus throughout the synapsis stage. It always stains more deeply than the linin. The mass of linin, therefore, lies between the nucleolus and the nuclear membrane. The later stages of synapsis are marked by the same striking peculiarities that were met with in Convallaria (*fig. 30*). In just the same way large globules of some deeply stainable matter accumulate on the outside of the contracted mass, appearing as if expelled from it. They seem, however, to be distinct from the nucleolus, as there is no apparent fragmentation nor budding of the latter. The nucleolus throughout the whole process remains of exactly the same size and regular contour, and with the little wart-like attachment undisturbed.

The spirem stage is much shorter than in Convallaria. The contracted linin network gradually begins to expand until the threads are again spread out beneath the membrane. Here again we notice a decided change in the structure of the linin thread, just as was the case in Convallaria. It is no longer so slender, and provided with such large granules, nor is it so conspicuously in the form of a network. The spirem is composed of a few rather thick linin threads extending in various directions around the nucleolus, and crossing each other occasionally. In them appear small chromatin granules, which, however, are much smaller and more regular in size than those present before synapsis (*fig. 31*). The whole process, therefore, is exactly comparable with that in Convallaria.

FIRST NUCLEAR DIVISION OF THE MOTHER-CELL.

The stages in the nuclear development preparatory to the first nuclear division are not nearly so marked as in Convallaria. The first indication of division is found in the gradual massing together of the chromatin into a number of irregular masses simulating those found just before synapsis. The difference lies in their larger and more equal size. Just at the time when the nuclear membrane is disappearing the number of these masses of chromatin may be determined approximately. Probably fourteen or sixteen is the correct number. They soon seem to lie together in pairs, in which case two may be easily mistaken for one (*fig. 32*). During the later stages the two parts of each pair seem to lose their identity, so that when on the spindle it is not possible to count more than seven or eight chromosomes.

Whether a fusion takes place here one cannot determine, since the small size renders it impossible to follow the process accurately. The exact manner of segmentation upon the spindle is also still in doubt. Seven chromosomes are found in the daughter nuclei, which leads us to infer that each of the original masses splits into two, but the preparations show no indications of any such division farther than that in many cases under high magnification, it seemed as if the chromosomes possessed a +-like structure similar to that in Convallaria, but the figures were not distinct enough to allow of any definite conclusions.

In regard to the formation of the spindle a few notes may be given, although the results do not differ essentially from those obtained by Mottier in Lilium. The kinoplasm is at first limited to a thin felt-like coat surrounding the nucleus (*fig. 32*). On account of the very large space occupied by the nuclear sap, it is easy to observe the entrance of the kinoplasm into the nuclear cavity. This takes place apparently before the entire disappearance of the membrane. The latter sometimes is still visible after the nuclear cavity is nearly filled with kinoplasm. At first thought it seems impossible to conceive of a substance passing through the nuclear membrane in this way. But Mottier[21] has shown that the membrane itself is probably nothing more than a close weft of kinoplasm. We have then merely to assume that the inner threads of this weft separate from the rest and traverse the nuclear cavity instead. Finally the whole membrane is entirely transformed into radiating threads. Thus it is not necessary to conceive of the kinoplasmic threads penetrating the membrane. They are from the first a part of it. At first the spindle is multipolar (*figs. 33, 34*), but the poles are few in number and very soon disappear, thus giving place to the normal bipolar type. In its mature condition the spindle is narrow and the poles are very acute (*fig. 35*). The fibers are few in number, probably not exceeding the number of chromosomes.

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[21] *Op. cit.* Pringsh. Jahrb. f. wiss. bot. **30**: 176. 1897.

In many cases the point at the pole toward which the spindle fibers converge was occupied by a granule both in the first and second division spindles. This granule in well-stained preparations was always dark, but its inconstant occurrence was decidedly against its being considered a permanent structure. The cell plate forms before the spindles of the second division (*fig. 36*).

The body, which looks so much like a nucleolus, disappears previous to the first division at almost the same time as does the nucleolar membrane. At this period it presents a more or less irregular and lobed appearance, but vanishes so quickly that it was impossible to determine whether the process was one of fragmentation or solution.

SECOND NUCLEAR DIVISION OF THE MOTHER-CELL.

Before the second division there is a distinct resting stage. An indistinct membrane is formed, and even a nucleolar body may appear. This latter, however, never becomes so large as in the archesporial nucleus, and often seems to be entirely absent, or at least indistinguishable from the chromosomes (*fig. 36*). A thick linin thread is usually formed, but the chromosomes remain distinct. In this character Potomageton agrees well with Convallaria. During this resting stage it is again possible to count the chromosomes, when the number is still found to be seven or eight. This resting nucleus can be distinguished easily by its much larger size from the one formed after the second division.

The origin of the spindle could not be traced, but many preparations showed it in the mature condition (*fig. 37*). These spindles are smaller and more slender than those described above. Like the latter, they have very pointed poles, and in both cases the poles are almost if not quite in contact with the cell wall.

The chromosomes are closely aggregated in the nuclear plate stage, so that it was impossible to determine just what happened to them at this point. The daughter chromosomes move to the poles very evenly, that is, with the same degree of rapidity (*fig. 38*). A count here showed the seven segments present. Although the daughter nuclei are quite small, the chromosomes remain distinct for some time, and can be again counted. After a time the nucleolar bodies also reappear, and the division is then complete.

THE MICROSPORE.

Our knowledge of the internal structure of the pollen grain really dates from the time of Hartig.[22] In this author's work is the first mention of the discovery in Tradescantia and several other plants of two nuclei in the pollen. This important discovery seems not to have been noticed by subsequent investigators until Strasburger's exhaustive work appeared in 1877.[23] It was not until then that the fact was generally recognized that two nuclei are to be found sooner or later in the development of every angiospermous microspore. This author was also able to demonstrate that the larger of these two nuclei is to be considered as a vegetative or prothallial nucleus and the smaller a generative nucleus.[24] Strasburger found also that a second division takes place regularly in angiosperms, either in the spore itself or in the pollen tube just before fertilization. Two sperm cells are thus formed from the one generative cell.

Since that time many investigators working upon widely different plants have found two nuclei, and these observations all lead to one result, namely the confirmation of Strasburger's observations in every essential particular. The time of formation, ultimate shape of the generative cell, and the time when the latter divides were indeed not always the same in different plants; on the contrary, all degrees of variation were found, some of which are noted below.

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[22] Botanische Untersuchungen aus der physiologische Lab. Land. Lehrung. Berlin. herausg. Karsten 3: 294. 1866.

[23] Befruchtung und Zelltheilung 18, 1877.

[24] Befruchtungs Vorgänge bei den Phanerogamen 5, 1884.

The microspores of Potamogeton become separate immediately after the second division of the mother-cell. The anthers are at this time still quite small, the subsequent growth being in reality for the purpose of accommodating the increase in size of the pollen grains. The microspores at first have a thin, although distinct and homogeneous cell wall surrounding the cytoplasm, and a very large nucleus. The latter fills at least one fourth of the whole cavity of the cell (*fig. 40*). The limited amount of cytoplasm present at this time is decidedly much more homogeneous than in the mature pollen, and stains with the gentian-violet a uniform pale violet similar to that of the cell wall. For a short time after the wall of the mother-cell disintegrates the pollen grains are still held together by the remains of these walls. In fact they are as if imbedded in a ground mass of some viscid matter.

The young grains very soon begin to increase in size, but the cytoplasm does not keep pace. As a result, the latter at length is confined to the parietal layer, but with a considerable increase in thickness on the side where the nucleus is located. These stages occur when the embryo-sac is yet one-celled, and of course while the spike of flowers is still enclosed within the bud.

Just before the nucleus begins to prepare for division we find the following conditions: The cytoplasm is decidedly more granular, and stains more deeply with the orange. The large vesicular nucleus possesses a very distinct membrane. Lying close against this is the linin thread which is rather extensive for Potamogeton. The thread however is nearly destitute of chromatin. The nucleolar-like body is smaller than usual, and in some cases more than one may occur.

The first division of the microsporial nucleus takes place much earlier than in Convallaria, and while the whole spike is yet enclosed in the bud. The spores reach their full size before the division, and it is at this time that the exine first begins to show signs of the thickening which produces the very slightly roughened surface of the mature spore. Owing to the small number of chromosomes present, the spindles in the pollen grain are exceedingly minute and slender (*fig. 41*). The process of division here does not seem to present any new features. When preparing for division, the primary nucleus moves toward one side of the cell, so that the resulting spindle has one pole in contact with the cell wall. This pole unlike the free one, is not pointed; on the contrary, it is usually quite broad, so that the spindle fibers are attached to the wall over a considerable area. The spindle is quite dense and stains readily, but is composed of few fibers. After the chromosomes pass to the poles a distinct cell-plate forms, and is later followed by a definite membrane (*fig. 42*). The latter is arched in such a way as to cut off one daughter nucleus in a small lenticular cell, of which one wall is the wall of the spore itself. This is the so-called generative cell.

The cytoplasm henceforth occupies the greater part of the cell cavity. It gradually becomes filled with large bodies which stain purple with gentian-violet, and blue with iodine. They are in reality starch grains. A similar occurrence of starch in the pollen grain has been described in Naias by Campbell.[25]

After a short period of rest the generative cell begins to elongate, notwithstanding the fact that it still appears to be closely attached to the wall. The elongation produces an oblong cell, and is the first step in preparation for the second division. The chromosomes for this division are formed early. They can often be seen to occupy nearly the entire nuclear cavity, and so distinct are they in many cases that one may count them. The number here again is uniformly seven. The spindles were found in considerable numbers, one of which is seen in *fig. 43*. The spindle fibers are very coarse and apparently scarcely more numerous than the chromosomes. They seem to stain more easily than is ordinarily the case in this plant. The chromosomes are during this division exceedingly minute.

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[25] Proc. Calif. Acad. Sci. III. **1**: 16. 1897.

The cell plate is soon deposited, and divides the generative cell into two parts. The two daughter cells do not separate, but remain connected as a two-celled body during their entire stay in the spore. A more or less prominent constriction often occurs at the middle, but this does not seem to be constant (*fig. 44*). The pollen grain is always somewhat flattened, and since the generative nucleus is usually adjacent to the flat side, it is not possible to tell whether the latter remains attached to the wall after division. The difficulty was increased since the pollen escapes from the anther very soon after the second division.

An examination of the literature relating to the pollen grain of the monocotyledons furnishes some interesting facts. Strasburger found the division of the generative cell to take place within the spore only in Juncus and Arum; while in all other cases the division was in the tube.[26] All these cases belong either to the Liliaceæ, Orchidaceæ, Amaryllidaceæ, or Iridaceæ. Schaffner found the division occurring in the spores of Typha, Alisma, and Sagittaria,[27] and Campbell found the same to be the case in Naias, while the writer finds the same phenomena in Acorus and Potamogeton. In all cases among the monocotyledons, where division occurs in the pollen grain, with the exception of Alisma and Sagittaria, the generative cell is at first enclosed by a wall, and always becomes two-celled after division, although Campbell claims that the two cells in Naias separate before passing into the tube. Schaffner was not able to discover any walls around the generative cell in the two above mentioned species. From this it appears that the division of the generative nucleus in the tube is mostly confined to the liliaceous and orchidaceous groups among the monocotyledons, while the division within the spore characterizes the spadiceous and naiadaceous groups.

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[26] Befruchtungsvorgänge bei den Phanerogamen 22. 1884.

[27] A contribution to the life history of *Sagittaria variabilis.* BOT. GAZ. 23:252. 1897.

SUMMARY.

The following brief summary may aid in bringing together the results reached in the foregoing pages.

The experiments with regard to the effect of external conditions on nuclear division both in Convallaria and Potamogeton gave no results for light and humidity, which were the only conditions tested.

The material illustrating the younger stages in the development of the microsporangium shows that the process is slightly different in Convallaria and Potamogeton from the normal method as given by Warming and Engler. The archesporial cells arise by the division of a hypodermal cell at one corner of the anther. Therefore, instead of the archesporium arising from a layer of hypodermal cells, as Warming describes for dicotyledons, it arises from one or rarely two hypodermal cells. The primary archesporial cells divide only a few times, but there is considerable subsequent growth in size of each cell. The next outer cell in the original row forms part of the tapetum, and the remainder are wall cells. Most of the wall and tapetum, however, is formed from the tissue at either side of the archesporium and in its rear. This differs from Warming's views mainly in the restriction of the hypodermal cell and in the derivation of the wall from the adjacent tissue. The anthers of all other monocotyledons which the writer has had an opportunity to examine seem to show that this is more likely the normal process for the whole group.

The tapetal nuclei of Convallaria show nicely the process of nuclear fusion which has been described by Strasburger and others for many other plants. After the division of the primary tapetal nucleus by the mitotic method, the two daughter nuclei in many cases fuse again, and all stages of the process may be found often in the same anther. It is probable that not all the nuclei divide, and also that not all of those that do divide fuse again before disintegration. It seems that in Potamogeton no division of the tapetal nuclei takes place.

The structure of the wall in the mature microsporangium was found to agree with the monocotyledonous type in general. The sequence in development was centrifugal and resulted in a well-defined endothecial layer, together with two or three inner wall layers in addition to the epidermis. At maturity only the epidermis and endothecial layer are present.

The development of the archesporial nucleus shows some very important features. The contracted condition called synapsis is without doubt a normal process accompanied by radical changes in the chromatin thread. The latter, which before synapsis was in the form of a network in which were imbedded large irregular chromatin masses, after synapsis is thicker, coil-like, and with the chromatin in smaller more equal masses. The spirem therefore begins at the close of the synapsis stage. In both plants studied irregular dark masses were apparently expelled from the chromatin thread at the close of the synapsis, and in Potamogeton at least it was plainly evident that these had no connection whatever with the nucleolus. The ultimate fate of this chromatin-like matter was not determined. Whether this phenomenon was artificial or natural could not be determined from the material at hand.

The growth and segmentation of the spirem in Convallaria is almost identical with that in Lilium as described by Mottier. The longitudinal splitting of the ribbon is especially noticeable. In Potamogeton the process is different but could not be worked out satisfactorily owing to the minute size of the nuclei. In this plant sixteen chromatin masses were counted just before division, but later there were only about seven, seeming to indicate a fusion of the widely separated primary segments to form the chromosomes. The number of chromosomes after reduction was eighteen in Convallaria and seven in Potamogeton. This last number is one of the smallest so far recorded for the phanerogams.

Spindle formation in all three plants agrees in every essential particular with the process described by Strasburger and Mottier for Lilium. The multipolar condition was evident in each case, but was less distinct in Convallaria.

The splitting of the chromosomes in the heterotypic division was in Convallaria exactly similar to that in Lilium. All the stages were especially clear. The + formation, however, began in some cases during the early multipolar condition. The process in Potamogeton was probably also normal.

Nothing new could be determined in regard to the segmentation during the second division. It seemed to be absolutely impossible to determine in these plants with any degree of certainty whether the division was transverse or longitudinal. All the phenomena, however, seemed to indicate a transverse rather than a longitudinal division in both plants.

The walls of the mother-cells in Potamogeton were thin as in Naias and Zannichellia.

In the microspores of Convallaria the generative nucleus is very chromatic, and is cut off by a distinct wall, but does not become detached from the wall of the spore until just previous to the time of passing into the tube. The division of the nucleus is probably in the tube since it was not found within the spore. As in Convallaria, so also in Potamogeton the generative cell is cut off very early, but in the latter plant the two sperm cells are immediately formed. The two male nuclei are inclosed each within its own cell wall, but they both still remain attached to the wall of the spore. The two-celled body then passes down the tube and even into the egg without separation of the two cells. The spindles in each case are very small and the chromosomes very minute.

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EXPLANATION OF PLATES XXIV–XXV.

PLATE XXIV. Convallaria majalis L.

FIG. 1. One of the angles of a young anther in cross-section, showing the row of cells derived from the primary hypodermal cell; the two inner are doubtless archesporial cells; the third will form part of the tapetum.

FIG. 2. An anther cell at a later stage; the two archesporial cells in *fig.* have divided several times forming a small mass of tissue; the row of wall cells at the top is probably equivalent to that in *fig. 1*; the tapetum is not yet formed.

FIG. 3. A section of the anther wall at a still later stage; on the extreme outside is the epidermis; then two layers the inner one of which will later disintegrate; the fourth layer is the tapetum the cells of which often contain two nuclei; and farther inside are shown a few archesporial cells.

FIG. 4. A portion of the anther wall at nearly the same stage as in *fig. 3*, showing the division of the tapetal nucleus.

FIG. 5. A slightly later stage of the same in which the daughter nuclei formed in *fig. 4* are in the process of fusion.

FIG. 6. A portion of the mature anther wall; the outer layer is the epidermis, the next below is the endothecium with spiral markings on the walls; and farther inside a disorganized mass composed of the tapetum and the one or two layers just outside.

FIG. 7. An archesporial nucleus in the resting stage; the linin network contains granules of chromatin of various sizes.

FIG. 8. Synapsis, the projection at the left is the nucleolus.

FIG. 9. Last stage of synapsis; the spirem ribbon is opening out, and between its meshes are chromatin masses of various sizes; the nucleolus at the left.

FIG. 10. The spirem ribbon with chromatin granules imbedded in the linin.

FIG. 11. The spirem ribbon after longitudinal segmentation, and cut in lengths by the section knife.

FIG. 12. Chromosomes still showing the double nature; dissolution of the nuclear membrane.

FIG. 13. The multipolar spindle with chromosomes in various views.

FIG. 14. The bipolar spindle and nuclear plate; the chromosomes from the end appear +-shaped, from the side view more elongated.

FIG. 15. The + separates into V-shaped segments which are seen moving toward the pole.

FIG. 16. During the resting stage before the second division, the nucleus viewed from the pole; the chromosomes remain distinct.

FIG. 17. The nuclear plate of the reducing division; the segments are so numerous that their form can be determined only with difficulty.

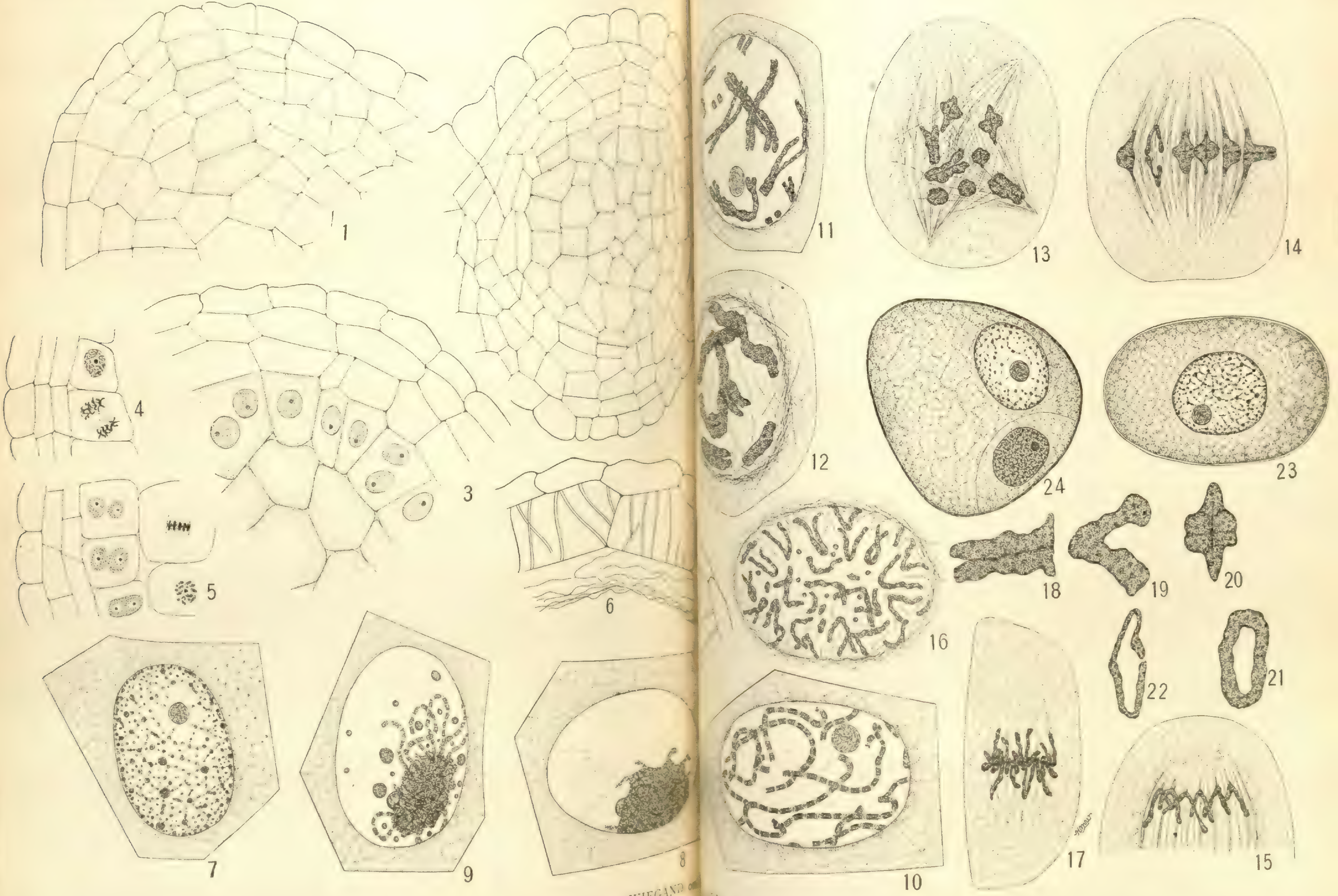
FIG. 18. A chromosome on the nuclear plate, side view.

FIG. 19. Pole view of a chromosome which is bent V-shaped.

FIG. 20. End view of *fig. 18*.

FIG. 21. A rare case, where *fig. 20* has opened out along the fissure line forming a ring.

FIG. 22. V-shaped segments ready to pass to the poles.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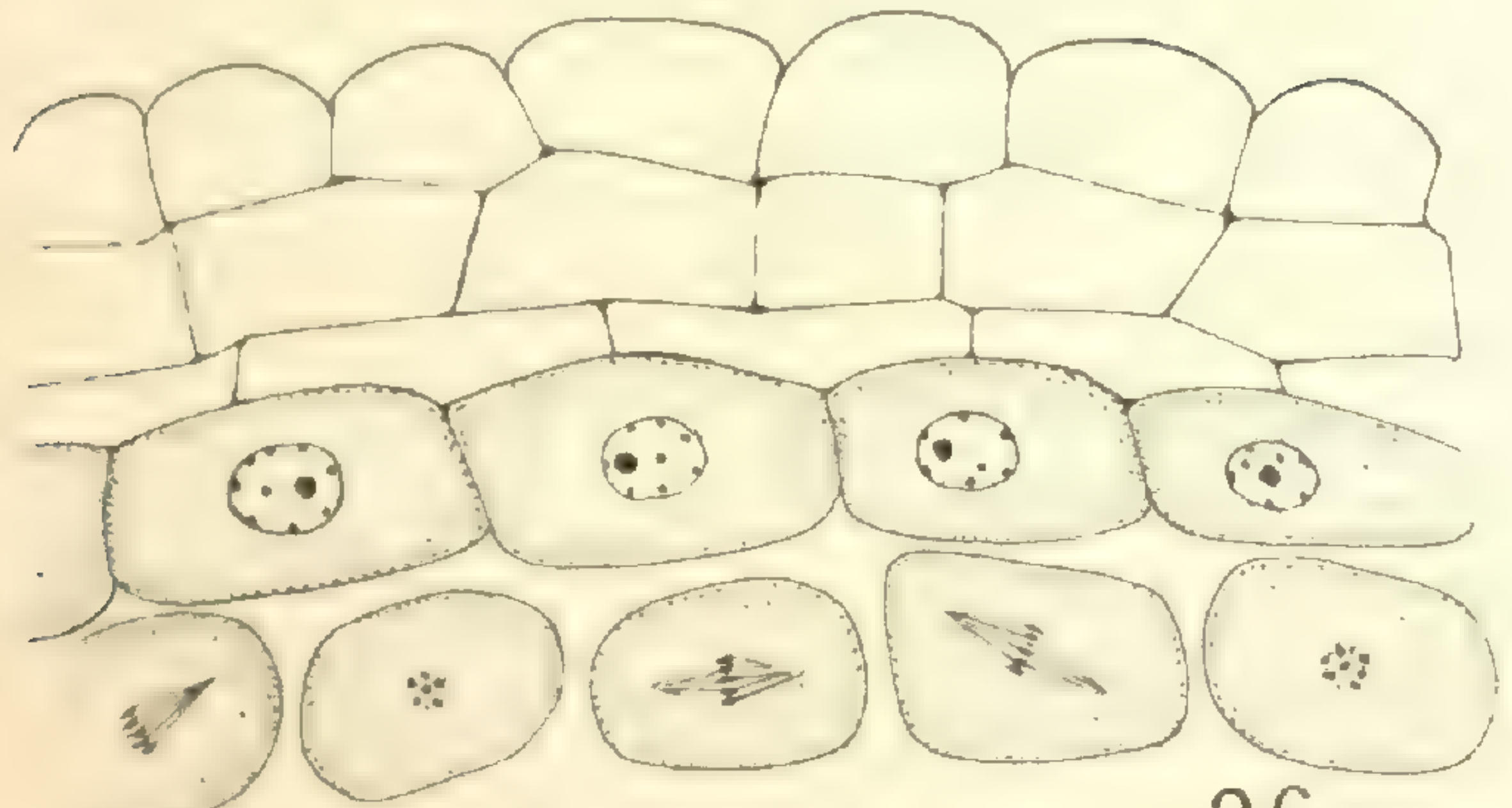

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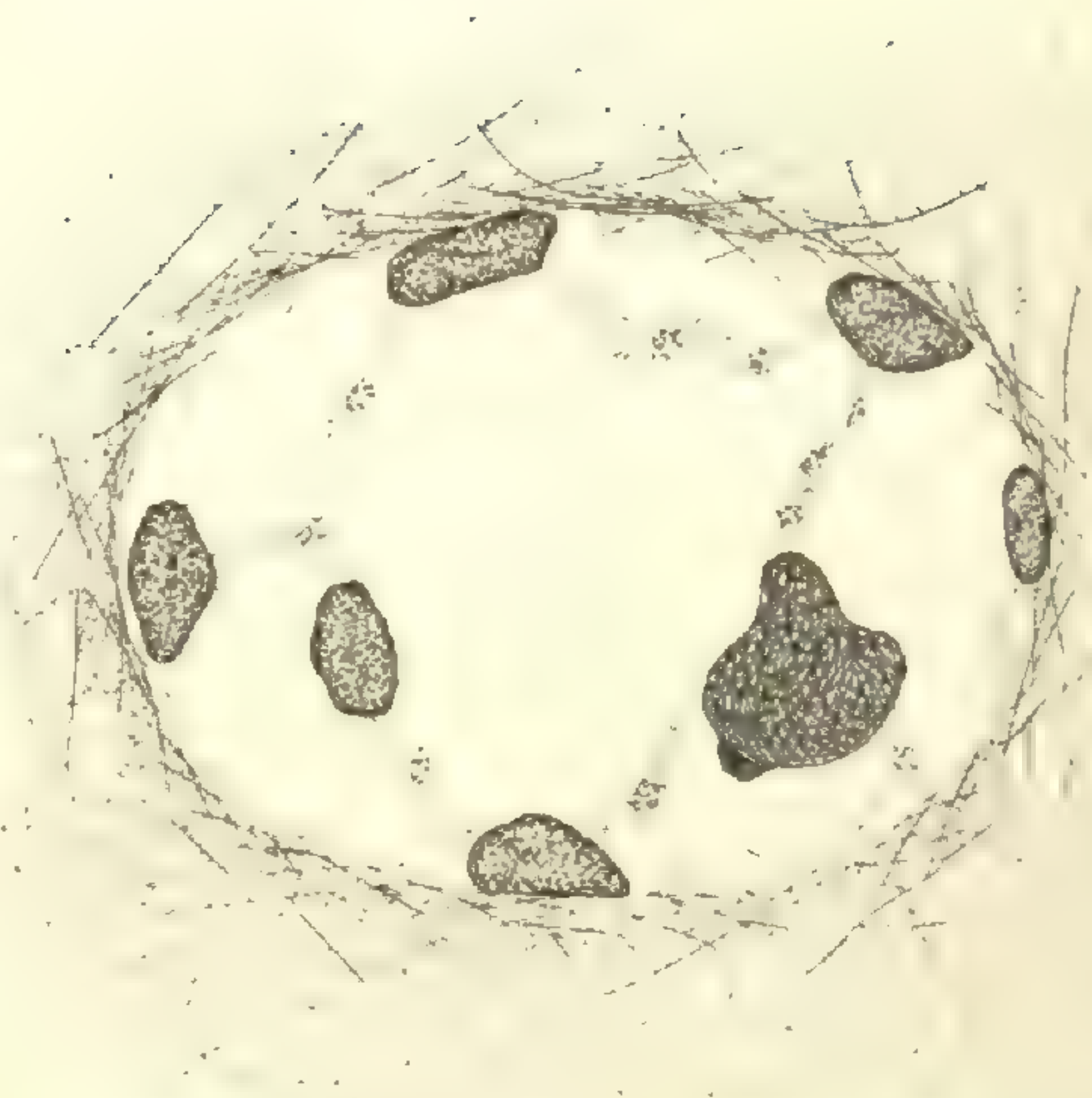

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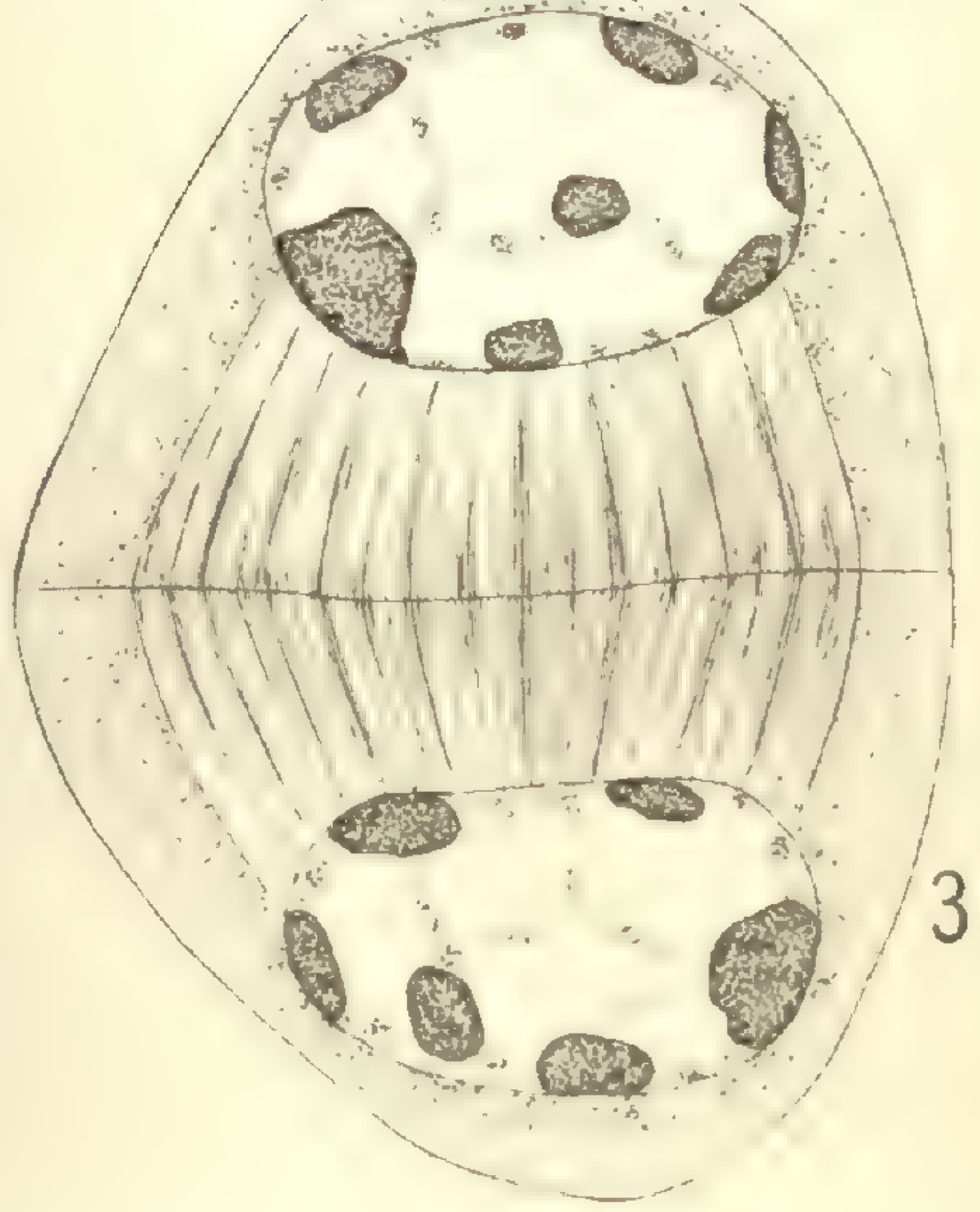

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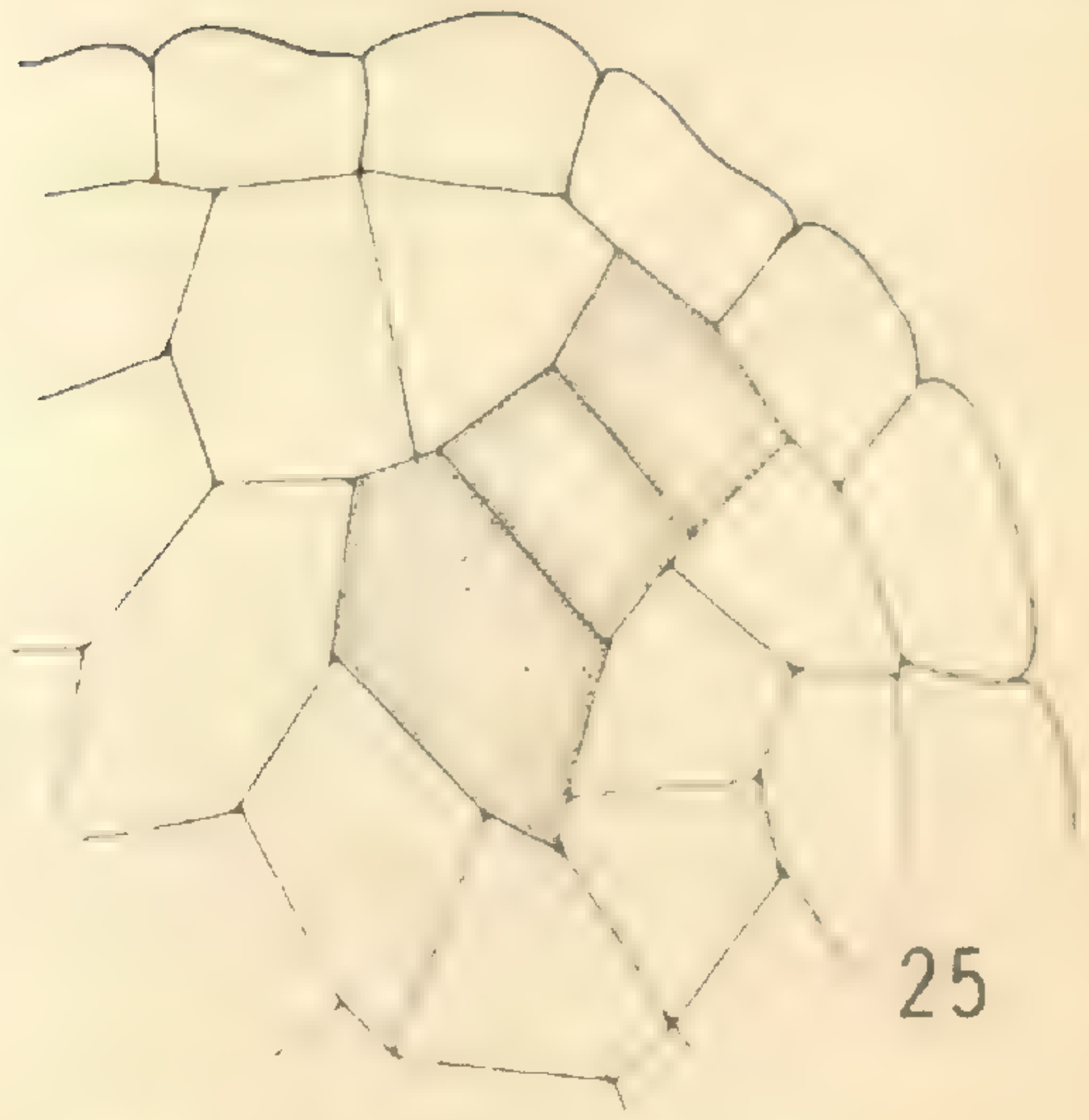

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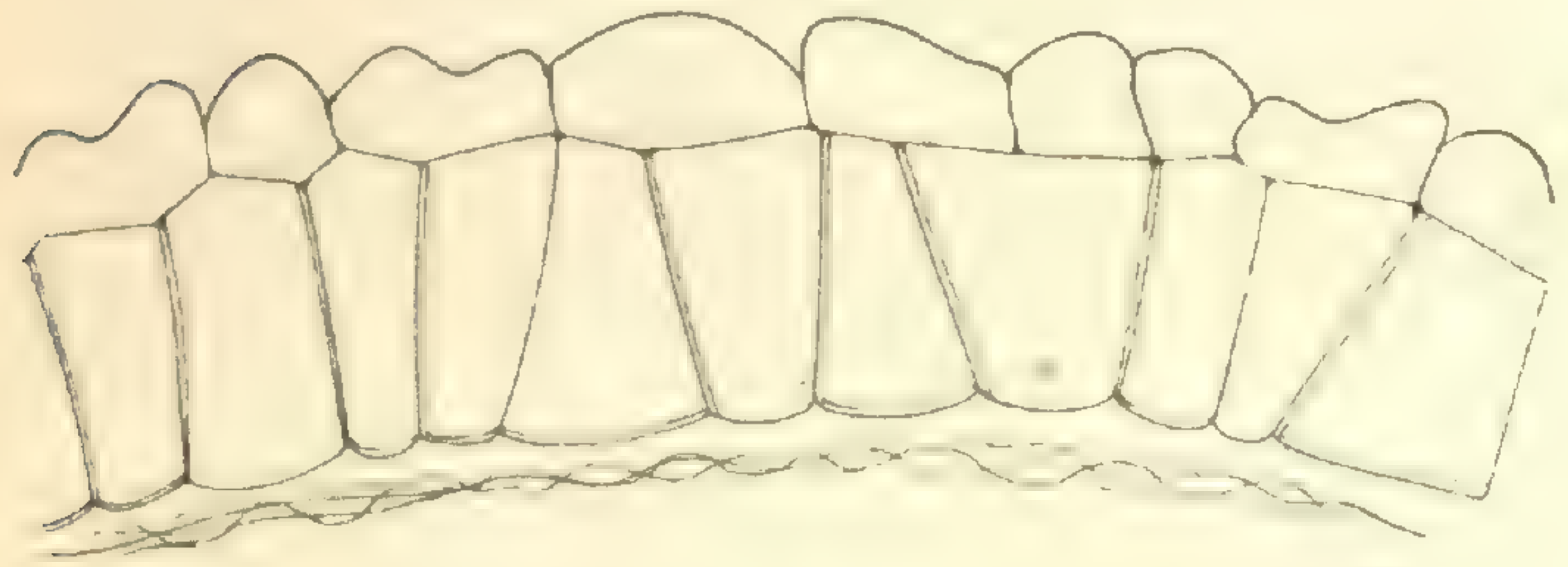

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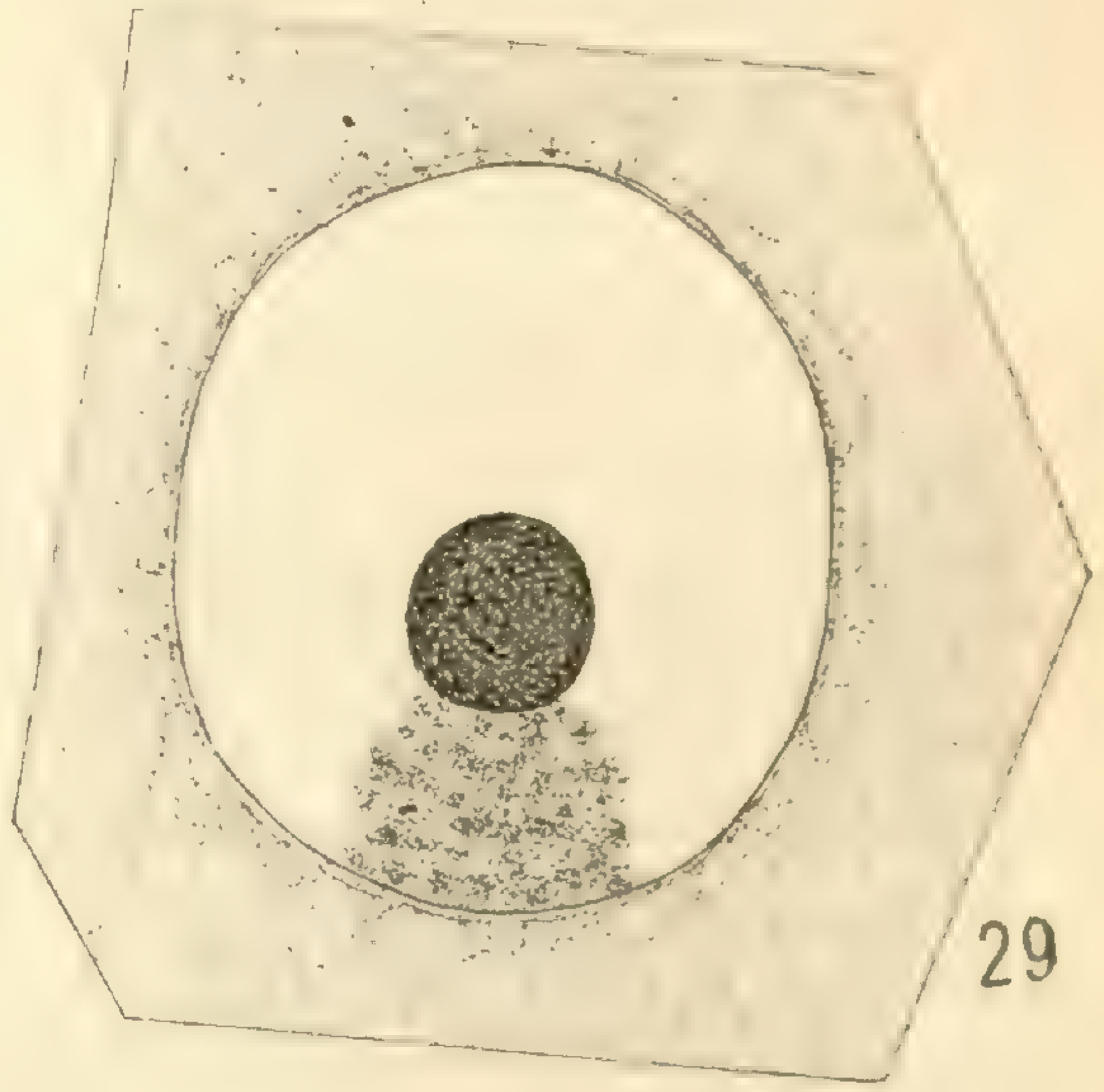

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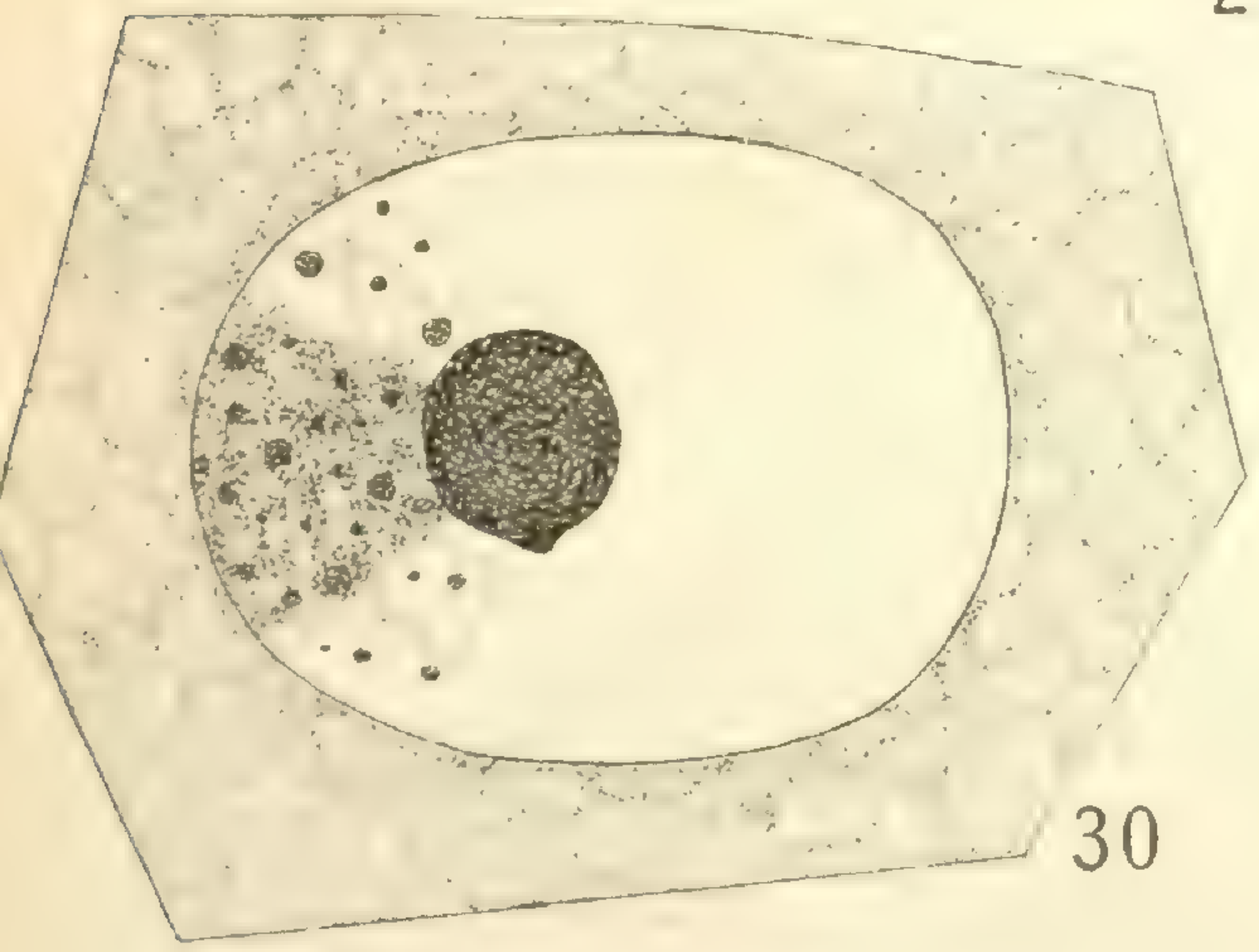

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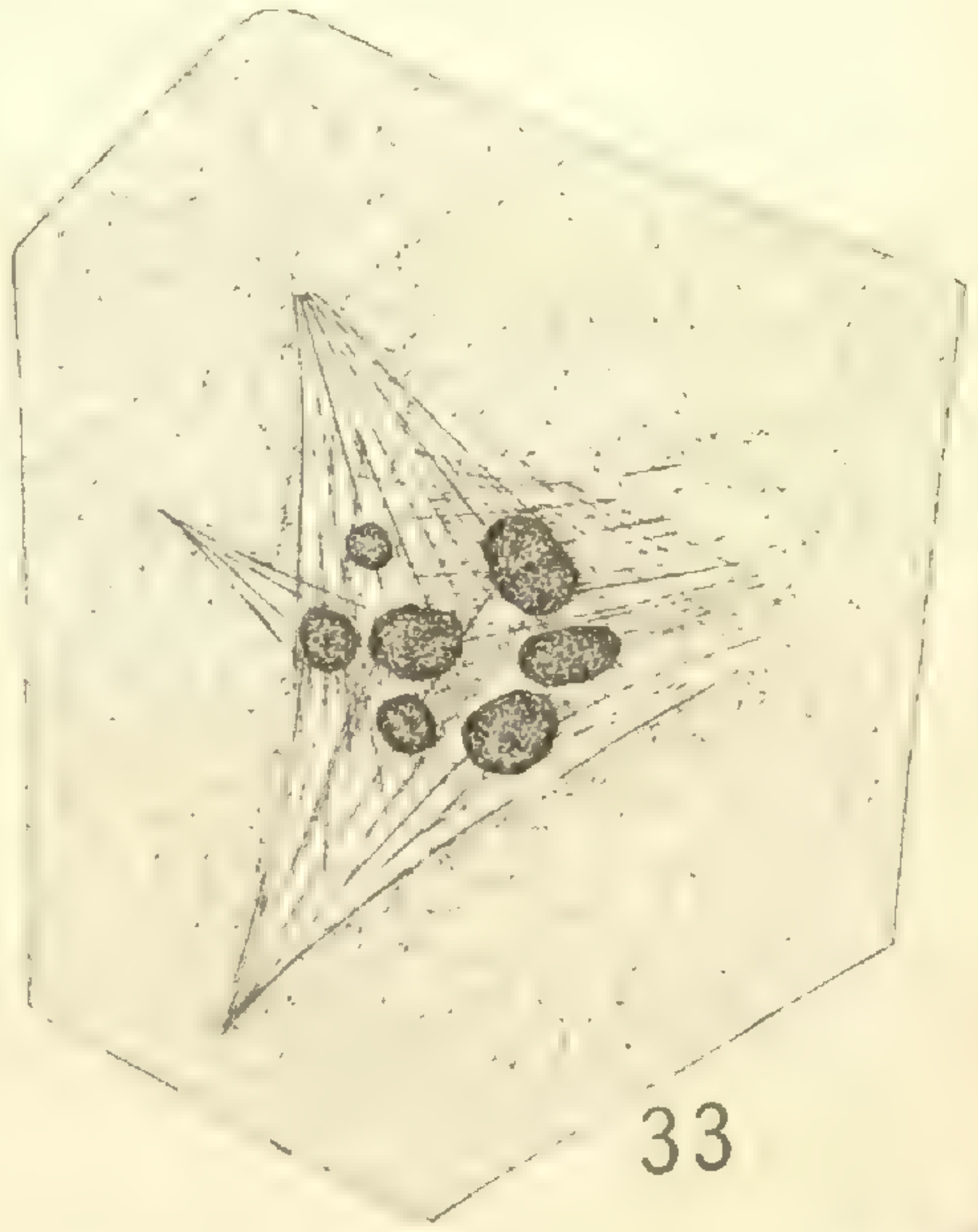

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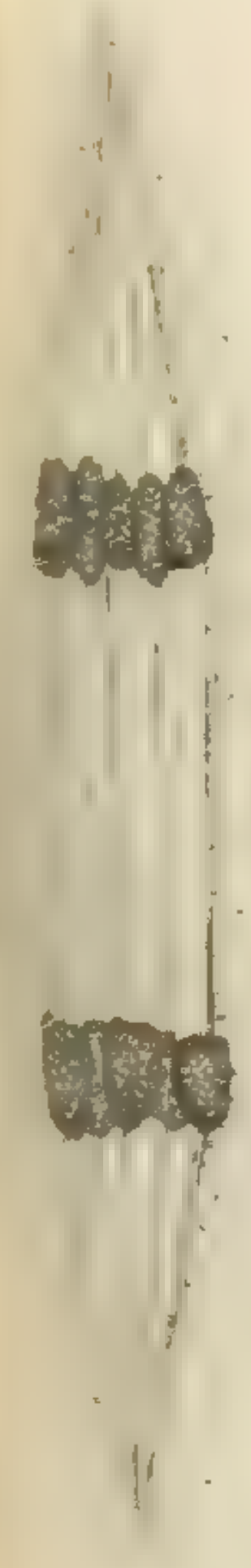

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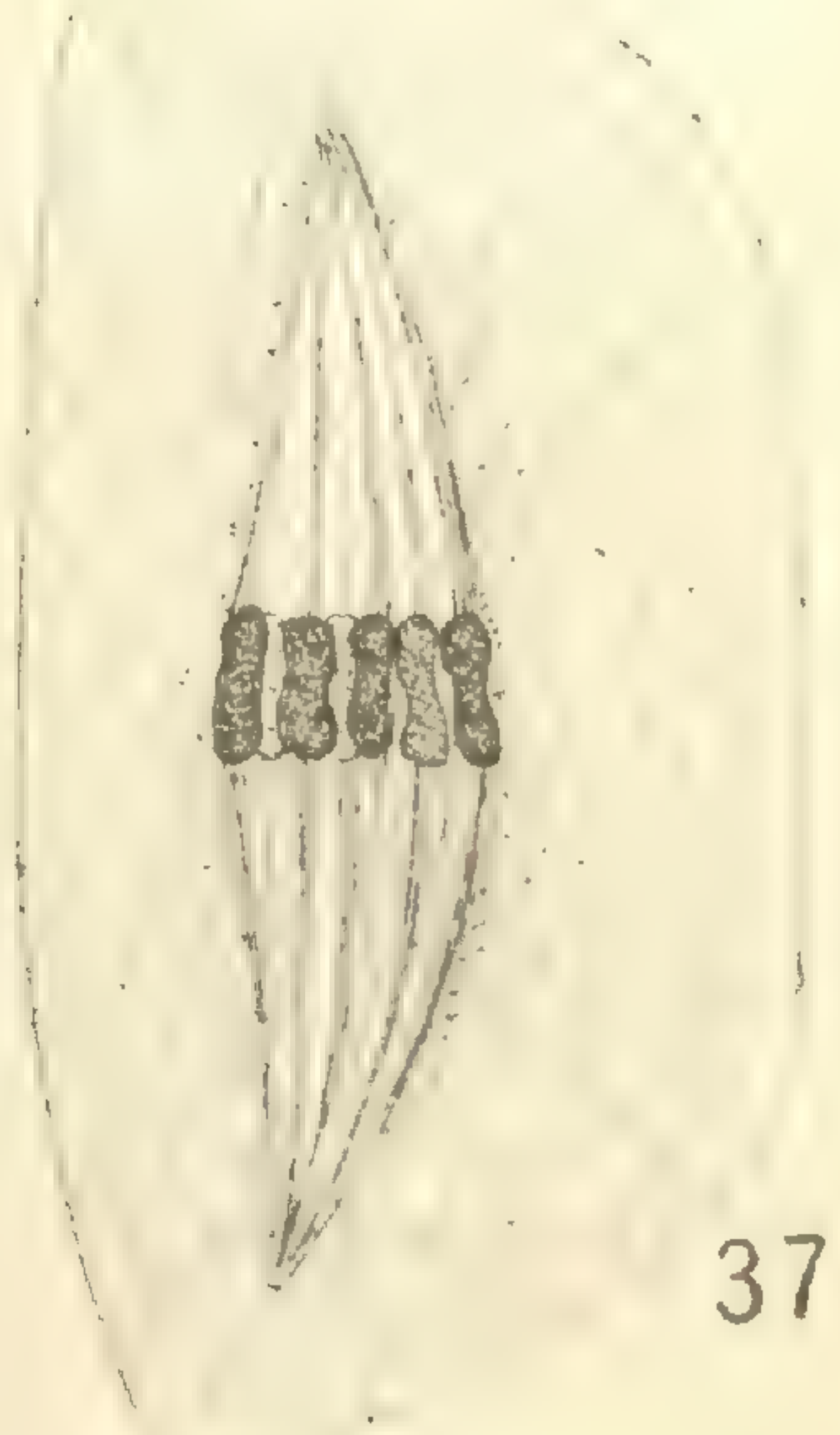

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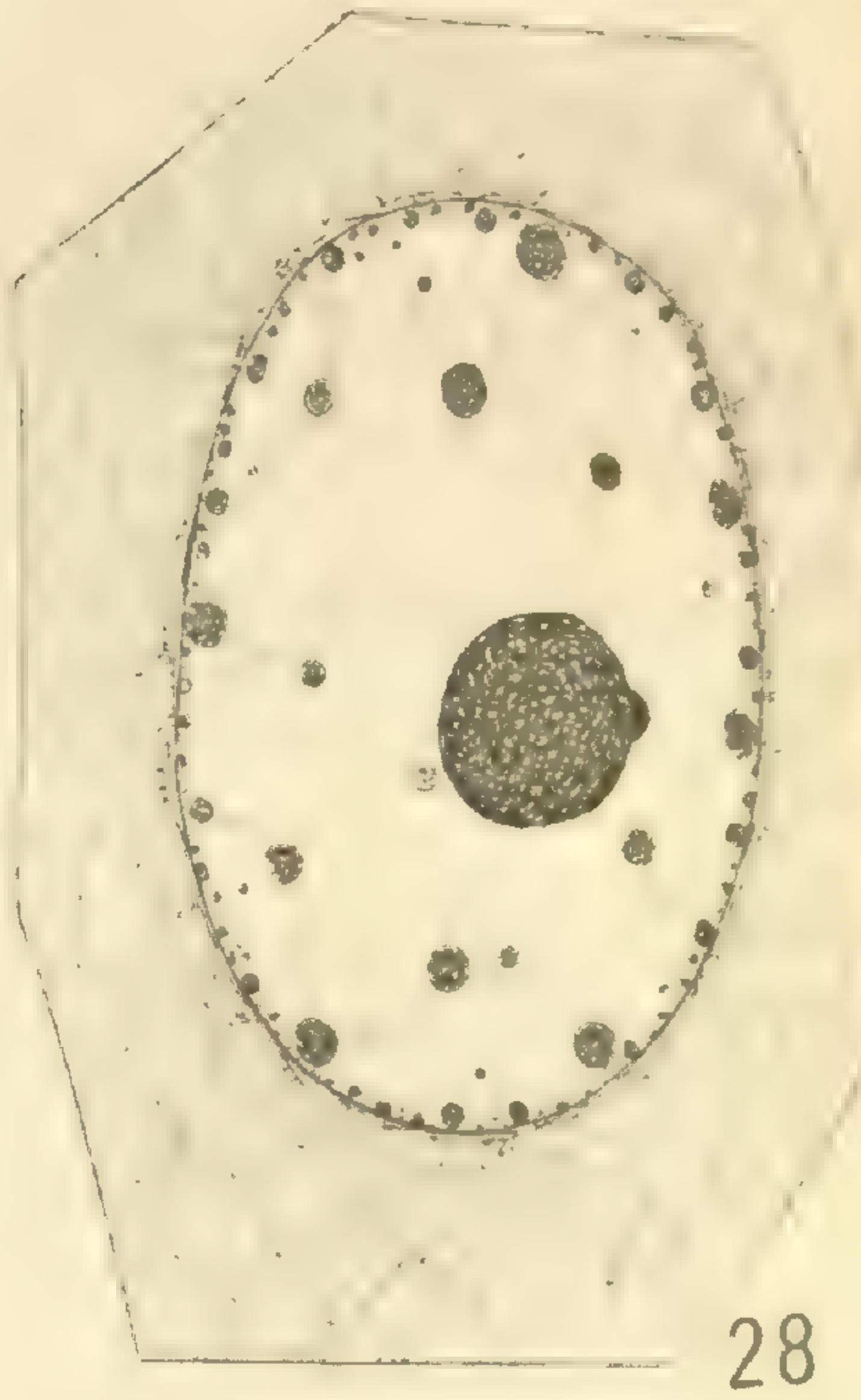

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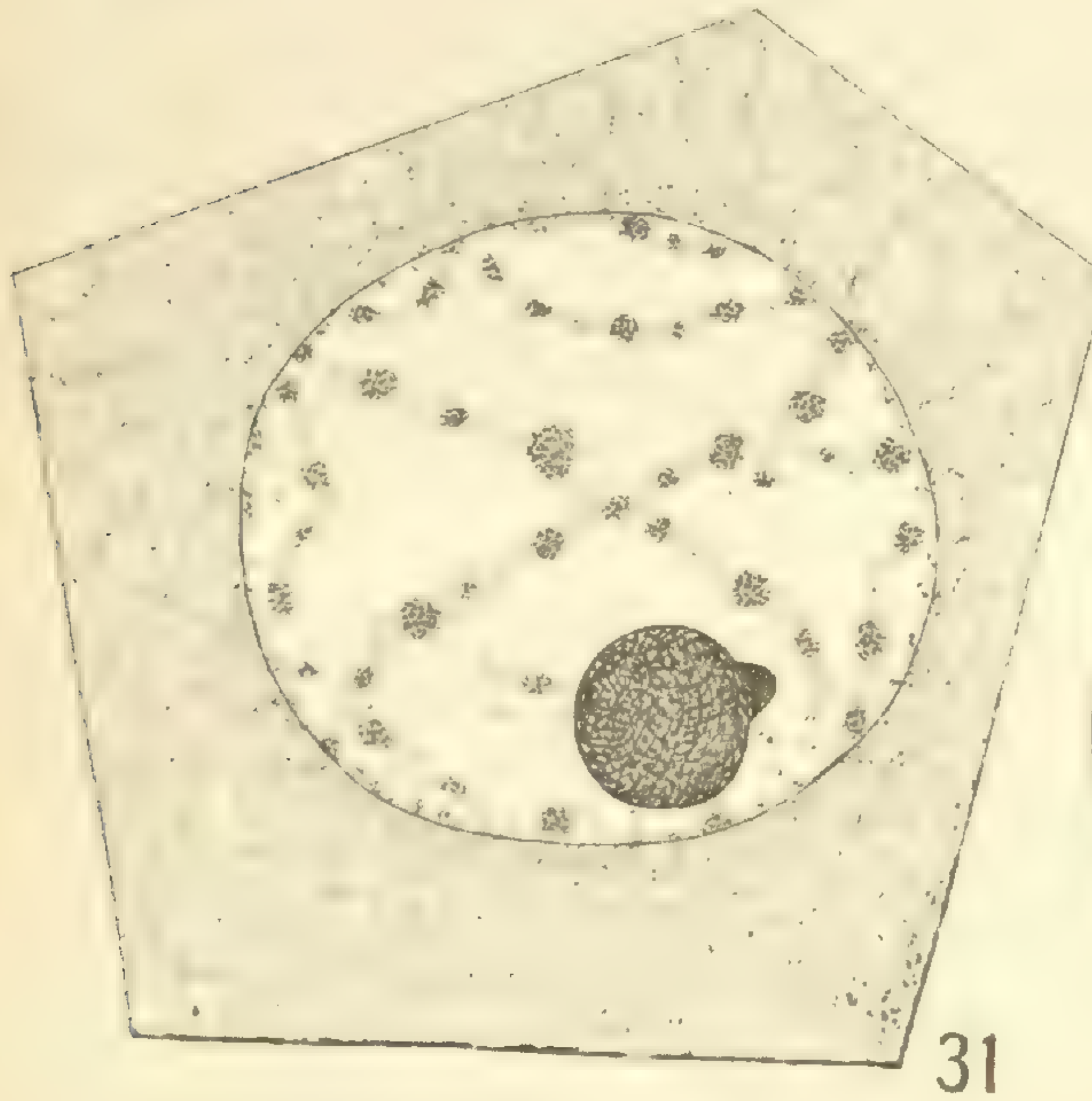

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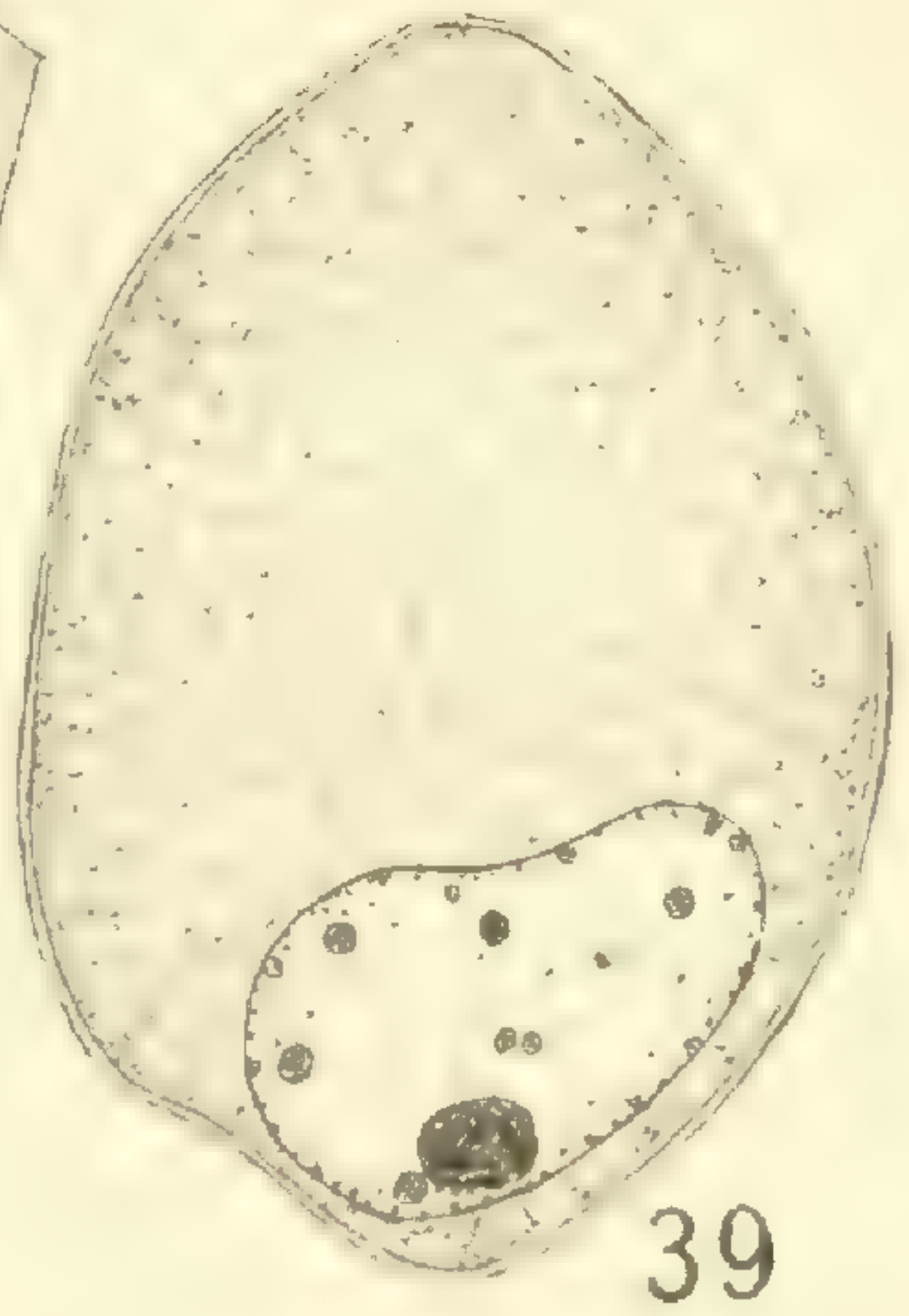

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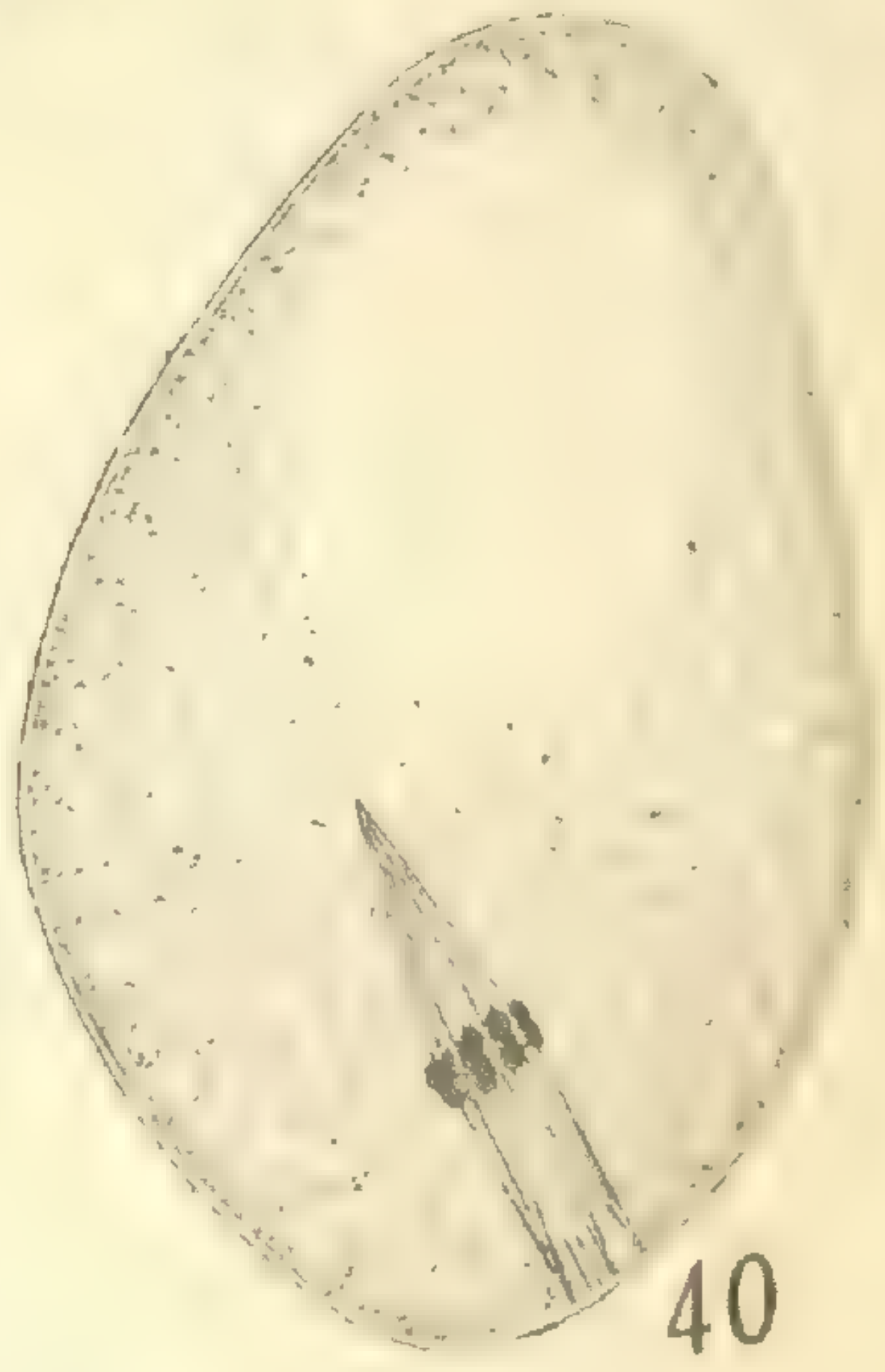

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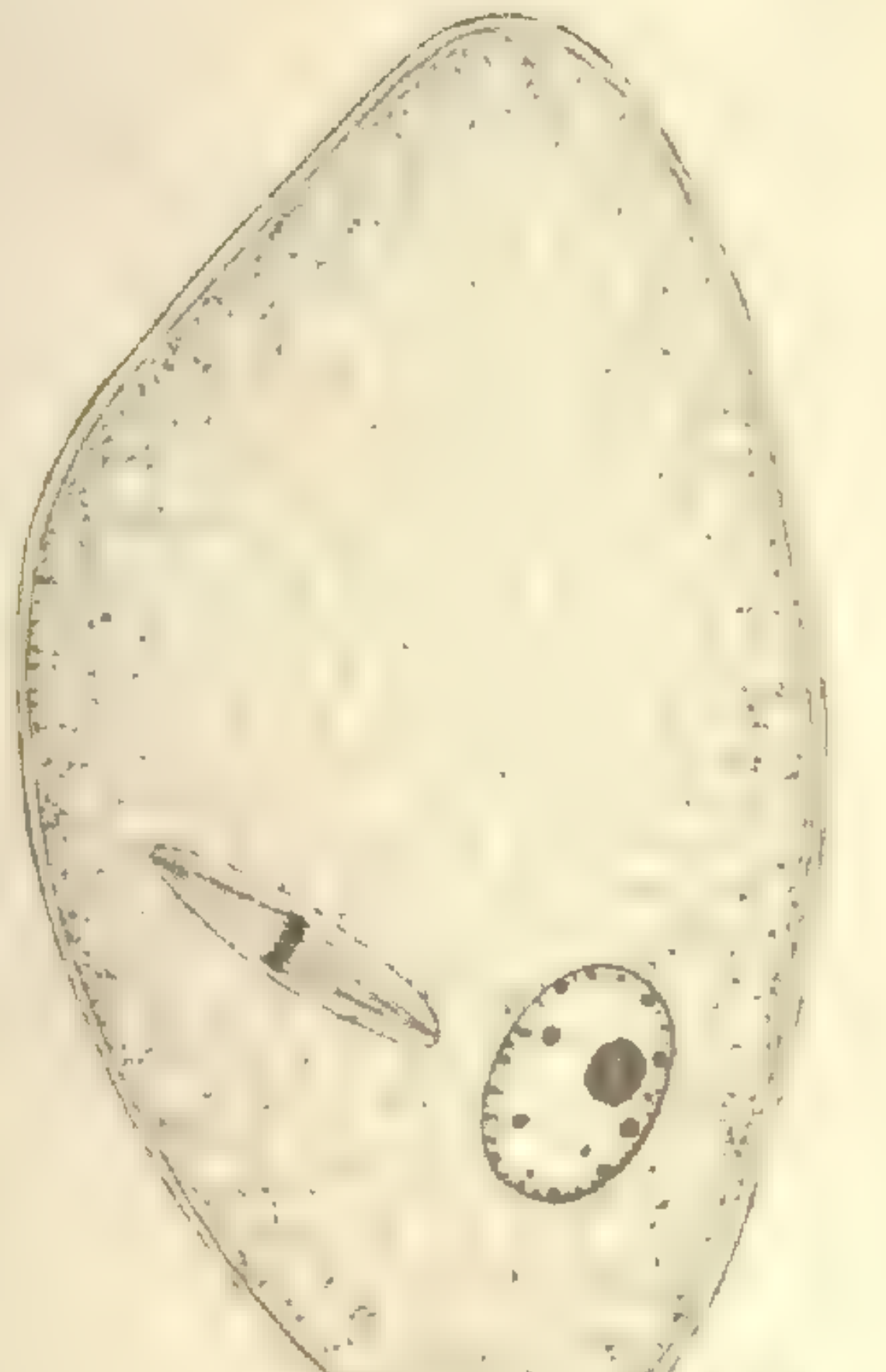

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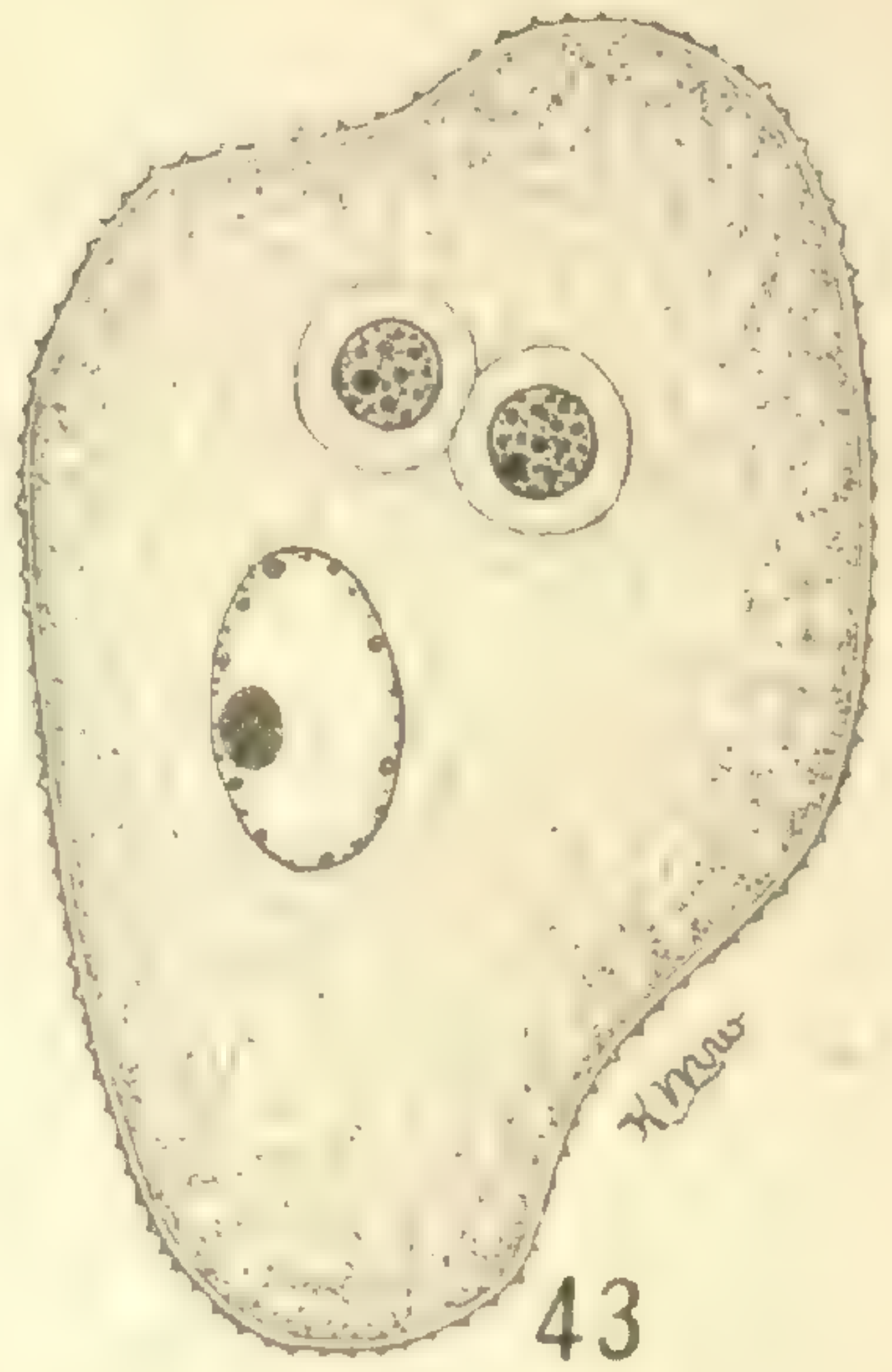

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FIG. 23. The microspore before the division of the primary nucleus.

FIG. 24. Same after the generative cell has been cut off; the generative nucleus is very dense.

PLATE XXV. Potamogeton foliosus Raf.

FIG. 25. The row of cells derived from the primary hypodermal cell at each corner of the young anther.

FIG. 26. A portion of the anther wall in a later stage; the outer layer is the epidermis, the second becomes the endothecium, the third disintegrates, the fourth is the tapetum, and farther inside are some archesporial cells.

FIG. 27. The structure of the mature anther wall; the endothecium with s indistinctly spiral markings is limited on the outside by the epidermis, and within by the disintegrated wall-cells and tapetum.

FIG. 28. An archesporial nucleus in the resting stage; the indistinct in network contains a few pale-staining chromatin granules; the large dark ss is the nucleolar-like body; and the black bud at the side is possibly the nucleolus.

FIG. 29. Synapsis; there is very little chromatin in the contracted mass.

FIG. 30. Later stage of synapsis; granules are expelled and the linin is beginning to spread out.

FIG. 31. The spirem; contains very little true chromatin.

FIG. 32. The nucleolar body has disappeared and at the same time the mosomes were formed; the membrane is here spreading out.

FIG. 33. The multipolar spindle and chromosomes.

FIG. 34. Same becoming bipolar.

FIG. 35. The bipolar spindle and nuclear plate; the chromosomes seem be double.

FIG. 36. Daughter nuclei after the first division; the chromosomes remain distinct.

FIG. 37. The second or reducing division, nuclear plate stage.

FIG. 38. Same showing the daughter segments moving toward the poles.

FIG. 39. The reducing division; chromosomes in the nuclear plate.

FIG. 40. A microspore with its primary nucleus.

FIG. 41. Same showing the mitotic division of this nucleus.

FIG. 42. Same with a free vegetative nucleus, and a small dense generave nucleus inclosed by a convex wall.

FIG. 43. Same showing the division of the generative nucleus.

FIG. 44. A mature microspore in which the two dense sperm nuclei are each enclosed by a cell wall.

BRIEFER ARTICLES.

AN HERMAPHRODITE GAMETOPHORE IN PREISSIA COMMUTATA.[1]

(WITH ONE FIGURE)

THE reproductive organs of most of the Marchantiaceæ are borne on specialized receptacles known as gametophores. So far as I can learn there has been no instance recorded of the two sex organs being found on the same gametophore. In a recent study of Preissia, however, I discovered this phenomenon.

The normal archegoniophore of Preissia is hemispherical in shape, the archegonia being situated on the lower surface. The tissue adjacent to the archegonia is compact and is made up of small cells rich in protoplasm. The tissue in the upper part, however, is much looser and the cells are larger. The antheridiophore is discoid, with the upper surface slightly concave, in which the antheridia are sunk. The tissue, particularly that surrounding the antheridia, is much looser than that of the female receptacle, and the cells are somewhat larger.

The shape of the hermaphrodite gametophore from which the accompanying illustration was made clearly indicates that it is primarily an archegoniophore, but it is modified to adapt it to its peculiar conditions. The upper part is more strongly developed than normally, and is irregular in form. The archegoniophore at maturity has a long stalk, but as the material from which this section was made was put up in the fall the stalk had not yet elongated. On the under surface is shown a portion of one archegonium, the neck having been cut off in sectioning. In the upper portion of the gametophore are two well-developed antheridia, corresponding almost exactly in shape and size to those occurring in normal plants. Their structure is also the same. Their position in the gametophore is very similar to that which they occupy in the normal antheridiophore. The tissue surrounding them resembles that of the latter receptacle, while the tissue of the lower portion is that of the ordinary archegoniophore.

---

[1] Miss Townsend discovered this interesting case of an hermaphrodite gametophore while engaged in the regular course of advanced work in comparative morphology, and it was at my suggestion that she has prepared it for publication.—GEO. F. ATKINSON.

There is a question whether this is merely an abnormal condition or whether it is a reversion to an earlier type. In the Ricciaceæ the two organs may either be produced together or, as is often the case with *Riccia glauca* and *Riccia hirta*, they may be found on the same part of the thallus, but occupying different areas, one organ being produced for a time and then the other. It is possible that in the evolution of the gametophore the tissue surrounding the reproductive organs may have become differentiated to form a receptacle, and, as the two organs were situated near each other, they may at first have been borne on the same gametophore. As the reproductive organs became more highly developed and specialized, the gametophore might naturally have advanced in development and specialization, and in time have come to produce but one kind of organ. A still further development might then have produced two distinct gametophores, each adapted in form and structure

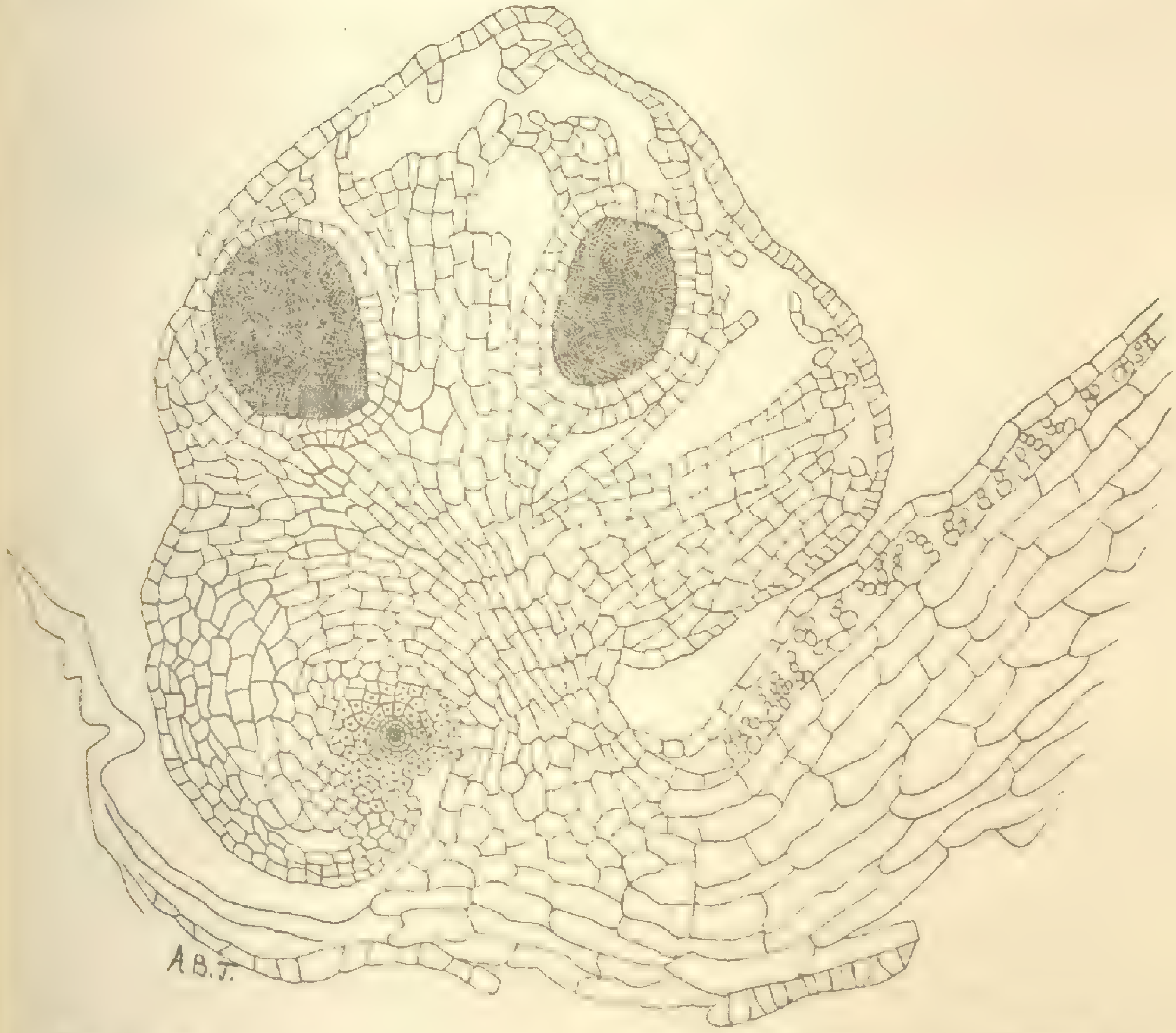

FIG. 1.—Section of hermaphrodite gametophore of *Preissia commutata*.

to the functions of the organ borne on it. A suggestive case in the algæ occurs in *Vaucheria geminata* and *V. terrestris*, where an unspecialized branch, or gametophore, bears both antheridia and oogonia. It is possible that the original type of gametophore was hermaphrodite. In *Vaucheria terrestris* there are occasional branches which bear but one organ, showing that a unisexual gametophore might be developed from the hermaphroditic.—ANNE B. TOWNSEND, *Cornell University*.

SOME PLANTS RECENTLY INTRODUCED INTO FLORIDA.

TWO YEARS since[2] I called attention to some South American species found by Mr. A. H. Curtiss in ballast or about streets in various parts of Florida. A package of specimens recently sent for study by Mr. Curtiss contains several South American and southwestern species apparently not before reported from Florida, most of them unrecorded from the North American continent. The plants from Pensacola were collected, in the words of Mr. Curtiss, "from lands lying inside the wharves, such as presumably consist more or less of ballast earth." This fact suggests that *Jussiaea suffruticosa*, and *Hydrocotyle bonariensis*, formerly reported from Pensacola, are perhaps introductions to be classed with *Alternanthera pungens*, *Solanum elæagnifolium*, and *Anthemis mixta* (recorded in the article referred to), and that they have reached the Florida coast through the same channel as the following species:

IPOMŒA PALMATA Forsk. Fl. Ægypt. Arab. 43.—This Egyptian plant has been recently introduced into Florida where it has made itself at home, growing vigorously in waste ground about St. Augustine, Jacksonville, and Pensacola (no. 6496). At Pensacola Mr. Curtiss states "I found it growing rampant over bushes on the bay shore and fruiting freely."

SOLANUM GLAUCUM Dunal in DC. Prodr. **13**[1]: 100.—A Brazilian species which was first noted at Pensacola in 1897 when it was not abundant, though now thoroughly at home (no. 6530).

SALPICHROA RHOMBOIDEA Miers in Hook. Lond. Jour. Bot. 4: 326. 1845.—This delicate solanaceous plant, native of Argentine Republic, was recently collected by Mr. Curtiss (Oct. 3, 1899) in low ground at Jacksonville (no. 6542).

[2] BOT. GAZ. **24**: 433–436. 1897.

Aster divaricatus Torr. & Gray, var. graminifolius Baker in Martius, Fl. Bras. **6**^{3}: 22.—This common South American form has been found for three years about wharves at Pensacola where it seems permanently established (no. 6497).

Erigeron bonariensis L. Sp. Pl. **2**: 863. 1753.—Growing luxuriantly (6 or 7 feet high) about the wharves at Pensacola (no. 6499). Introduced from South America.

Pascalia glauca Orteg. Hort. Matr. Dec. 39. This Chilian species has been introduced within a year or two at Pensacola (no. 6492).

Pectis prostrata Cav. Ic. **4**: 12. *pl. 324.* Very recently introduced at Pensacola (no. 6531): abundant from the southwestern states south through Mexico.—M. L. Fernald, *Gray Herbarium.*

CURRENT LITERATURE.

MINOR NOTICES.

LIEFERUNGEN 190–192 of Engler and Prantl's *Pflanzenfamilien* have recently been published. They are devoted to a continuation of the Polypodiaceæ by L. Diels.—C. R. B.

THE PAPERS of botanical interest printed in abstract or in full in the *Proceedings* of the Indiana Academy of Science for 1898 (published 1899) are as the following. THOMAS: Some desmids of Crawfordsville; MOTTIER: Nuclear division in vegetative cells; The centrosome in cells of the gametophyte of Marchantia; Endosperm haustoria in *Lilium candidum*; RISLEY: Absorption of water by decorticated stems; ARTHUR: Indiana plant rusts, listed in accordance with latest nomenclature; SNYDER: The Uredineæ of Madison and Noble counties, with additional specimens from Tippecanoe county; GOLDEN: *Aspergillus oryzæ;* CURTISS: A red mold; OLIVE: Affinities of the Mycetozoa; CUNNINGHAM: Morphological characters of the scales of Cuscuta; COULTER; Notes on the germination and seedlings of certain native plants; BRANNON: Some Indiana mildews.—C. R. B.

STATISTICAL methods have come into greater prominence in biological study during recent years. Dr. Charles B. Davenport has prepared a useful little handbook,[1] in which, after some preliminary definitions, he sets forth the proper methods of measuring and counting organisms, of seriation and plotting of data, describes the constants of plotted curves and probable errors in their determination, and enumerates the classes of plotted curves. A chapter is devoted to correlated variability and the methods of determining the degree of correlation and heredity. Galton's, Pearson's and Duncker's methods of determining the coefficient of correlation are given. Formulas, rules, and ten tables useful for the various calculations make up the bulk of the handy volume.

A short chapter on the applications of statistical biological study calls for remark. That these methods are of great value for a study of variation and heredity admits of no doubt. That they will improve our conceptions of species and varieties is not at all clear. That "by the use of the quantitative method biology will pass from the field of the speculative sciences to that of the exact sciences" is surely a vain hope. We shall see the pendulum swing far toward the side of quantitative study and then back to a point nearer equilibrium.— C. R. B.

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[1] DAVENPORT, C. B.: Statistical methods with special reference to biological variation. 12mo. pp. viii+148, *figs. 28*. New York: John Wiley and Sons. 1899. $1.25.

THE DEPARTMENT OF AGRICULTURE issues as one of its bulletins the first part of a useful little book, by the Chief of the Division of Forestry under the title, *A primer of forestry.*[2] In four chapters Mr. Pinchot presents in simple language (1) an account of the structure and activities of a tree as an individual; (2) the relations of trees in a forest, including their requirements as to light and moisture, and their reproduction; (3) the life of a forest, discussing the origin of the forest, the struggle between the trees, their death, and the modes of lumbering; (4) the enemies of the forest, including man, grazing, browsing, and trampling animals, insects and fungi, wind, snow, and fire.

The book is certain to be widely called for and is an excellent introduction to forest life for school reading. The physiology and anatomy are very elementary, but as accurate as such general statements can be. The numerous figures and plates which are almost all half tones from photographs, constitute an attractive feature: so much overdone, however, that half of them might be spared, as far as the text is concerned.— C. R. B.

THE LAST of the *Contributions* from the United States National Herbarium (5: 145–259. *pls. 18–64.* 1899) is one of the most notable of the series. It contains the results of the most recent studies upon the Mexican and Central American flora by Dr. J. N. Rose, whose untiring labor both in the field and in the herbarium has resulted in large accessions to our knowledge of Mexican and Central American plants. The *Contribution* consists of two distinct parts, viz., taxonomic studies, illustrated by text cuts and ten plates; and notes on useful plants of Mexico, illustrated by thirty-seven plates of unusual excellence.

The taxonomic studies are as follows: a rearrangement of the suborder Agaveæ, in which eight genera are recognized, *Pseudobravoa* being new; synopsis of the North American species of Nissolia, including twelve species, five of them new; notes on Rutaceæ, with two new species of Xanthoxylum; notes on Turneraceæ, with a new species of Turnera; notes on Mexican species of Clitoria, with descriptions of two new species; notes on Malvaceæ and Bombaceæ, with descriptions of ten new species; notes on Passiflora, with one new species; synopsis of the North American species of Waltheria, including ten species, three of them new; notes on some Mexican species of Thalictrum, six of them being new; Cedrela, or Spanish cedar, with descriptions of two new species; notes on new or rare Leguminoseæ, fourteen new species being described; descriptions of twelve miscellaneous new species.

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[2] PINCHOT, GIFFORD: A primer of forestry. Part I. The forest. 12mo., pp. 88, *pl. 47, figs. 83.* Washington: Dept. of Agric. 1899.

In addition to these Mexican studies a new genus of Commelinaceæ, *Treleasea*, is established with three species, to include certain Texan and Mexican forms heretofore referred to Tradescantia; and three new species of Tradescantia from the United States are described. A new genus of Umbelliferæ from Mt. Ranier, Washington, *Hesperogenia* by name, is described by Coulter and Rose; and Mr. L. F. Henderson describes a new Aster and a new Angelica from Idaho.

The part devoted to a description of the useful plants of Mexico is based upon the personal observations of Dr. Rose during a visit of four months in the summer of 1897. It is full of interesting information and photographic illustrations, and is very suggestive of lines of economic investigation.—J. M. C.

NOTES FOR STUDENTS

M. J. GOLDBERG'S experiments lead him to the conclusion that during the germination of wheat in darkness proteid substances are produced in the embryo in considerable quantity,[3] although Godlewski in 1897 thought this to be impossible.[4]—C. R. B.

COPELAND AND KAHLENBERG, by a series of carefully conducted experiments show that the injury to plants from solutions of pure metals (Nägeli's oligodynamic effect) is due to the toxicity of the compounds (salts) which the dissolved metals form and not to any peculiar or toxic action of the elemental metal.[5]—C. R. B.

M. W. PALLADINE has determined that alternations of temperature accelerate the respiration of severed tips of etiolated shoots of *Vicia Faba* cultivated in 10 per cent. cane sugar. The increased energy of respiration does not depend on the quantity of active nitrogenous foods, but the real cause is not yet determined.[6]—C. R. B.

DR J. W. HARSHBERGER has observed a distinct thermotropic curvature of leaf blade and petiole in *Rhododendron maximum* L.[7] In cold the blades are revolute and the petiole arcuate downwards. On bringing a branch into a warm room erection and flattening were complete within five minutes. The curvatures in a reverse direction are slower. Turgor variations are the cause.—C. R. B.

ITEMS OF TAXONOMIC INTEREST are as follows: GERRITT S. MILLER (Proc. Biol. Soc. Wash. **13**: 79–90. 1899) has discussed the species of Apocynum in the District of Columbia, recognizing seven, three of them being described as new, two having been recently described by Professor E. C. Greene, and the remaining two being the well-known species of Linnaeus.— WILLIAM PALMER (Proc. Biol. Soc. Wash. **13**: 61–70. 1899) has published a list of the ferns of the Dismal Swamp, Virginia, sixteen in number, one of them being described as a new variety.— J. N. ROSE (11th Ann. Rep. Mo. Bot. Gard. 1–5. 1899) has described a new species of Agave and critical notes on other species, accompanied by four plates.— E. P. BICKNELL in his further studies of Sisyrinchium (Bull. Torr. Bot. Club **26**: 335–349, 445–457, 496–499. 1899) has added eleven new species to the already long list of forms.— AVEN NELSON in continuing his publication of new plants from Wyoming (*ibid.* 350–358, 480–487) describes twenty-three new species, one of which represents a new genus, *Nacrea*, related to Anaphalis.— C. L. POLLARD (*ibid.* 365–372) has revised the genus Achillea in North America, recognizing ten species, three of which are new. — K. M. WIEGAND (*ibid.* 399–422) presents ten species of Bidens found in the United States and Canada, describing one new species and five new varieties.— ANNA M. VAIL in continuing her studies of Asclepiadaceæ (*ibid.* 423–431) discusses the types of Gonolobus and describes three new species of Vincetoxicum.— P. A. RYDBERG (*ibid.* 541–546) has described twelve miscellaneous new species from the western United States. —A. A. HELLER in continuing the publication of his new and interesting plants from western North America (*ibid.* 547–552) describes ten new species, four of which are species of Mertensia.— J. M. C.

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[3] Rev. gen. de Bot. **11**: 337–340. 1899.

[4] Anzeiger Akad. Wiss. Krakau, March 1897, *fide* Goldberg.

[5] Trans. Wis. Acad. of Sci. **12**: 454–474. 1899.

[6] Revue gen. de Bot. **11**: 241–257. 1899.

[7] Proc. Phila. Acad. Sci. **1899**: 219–224. *fig. 3*.

SOME CURIOUS experiments by A. Pagnoul[8] on transpiration are reported in the *Experiment Station Record* 11: 118. 1899. They are difficult to explain without more light than the brief summary gives; but to call attention to them we reproduce the abstract. "Experiments are reported in which fescue grass was grown from March 30 to June 21 under almost identical conditions, the only difference being that one pot was filled with a poor clay soil without fertilizer, and the other with a rich calcareous soil to which dried blood and nitrate of potash were added. The same degree of saturation of soil was constantly maintained. The grass was cut May 2, 27, and June 21, weighed and analyzed. The results obtained are tabulated. It appears that during the first period, 33 days, the plants in the poor soil transpired 1190^{gm} of water per gram of dry weight; as compared with a transpiration of 555^{gm} in the rich soil. In the second period the figures were 1053 and 581^{gm} of water per gram of dry weight, and for the last period 1084 and 585^{gm}, respectively. The nitrogen content of the product of each pot was determined, and it was found that for each gram of nitrogen in the product of the poor soil 46^{kg} of water was transpired, while in the rich soil 1^{gm} of nitrogen was found for each kilogram of water given off.

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[8] Bul. Sta. Agron. Pas de Calais **1898**: 10–15. *fig. 1.*

NEWS.

PROFESSOR DR. FRANZ VON HÖHNEL has left Vienna on a journey to Brazil.

SYNTHETIC cassia oil, introduced by Schimmel & Co. of Leipzig, is gradually displacing the ordinary cassia oil of commerce.

A PLANT of *Cypripedium insigne giganteum* from the collection of the late Major Mason, recently sold in London by auction, brought £147, and other varieties from £30 to £76. The total amount realized at the sale was over £3000.

THE HERBARIUM of the Geological Survey of Canada has recently been enriched by Professor Macoun's collections from Sable Island, by Mr. A. P. Low's from northern Labrador and the coasts and islands of Hudson's bay; by Mr. J. B. Tyrrell's from the Yukon district; and by Mr. N. B. Sanson's from the vicinity of Banff.

A COURSE for teachers, consisting of studies in ecology and plant physiology with excursions and practice, was conducted during the autumn by Professor C. Stuart Gager, of the New York State Normal College at Albany. The syllabus shows a refreshing appreciation of the modern aspect of botany, "considering plants from a dynamic rather than a static point of view."

THE ACREAGE in peppermint in Wayne county, New York, one of the chief centers of the crop, has fallen from 3340 acres in 1890 to 300 in 1899, on account of low prices of the oil and the better returns to farmers from growing sugar beets. In Michigan the exports of peppermint oil rose from 80,225 lbs. in 1894 to 162,492 lbs. in 1897, but are now falling, being 145,375 lbs. in 1898.

WITH THE CLOSE of volume II Messrs. Willard N. Clute & Co. cease to publish *The Plant World.* It will hereafter be published by The Plant World Company, 321 4½ street, Washington, D. C. The first number of volume III will be that for January 1900, thus leaving a gap from October to December 1899 inclusive. The journal still remains under the editorial direction of Dr. F. H. Knowlton. The new volume will be more fully illustrated and nearly doubled in size, but with the same subscription price. The journal has proved very useful, and we bespeak for it the fuller success it deserves.

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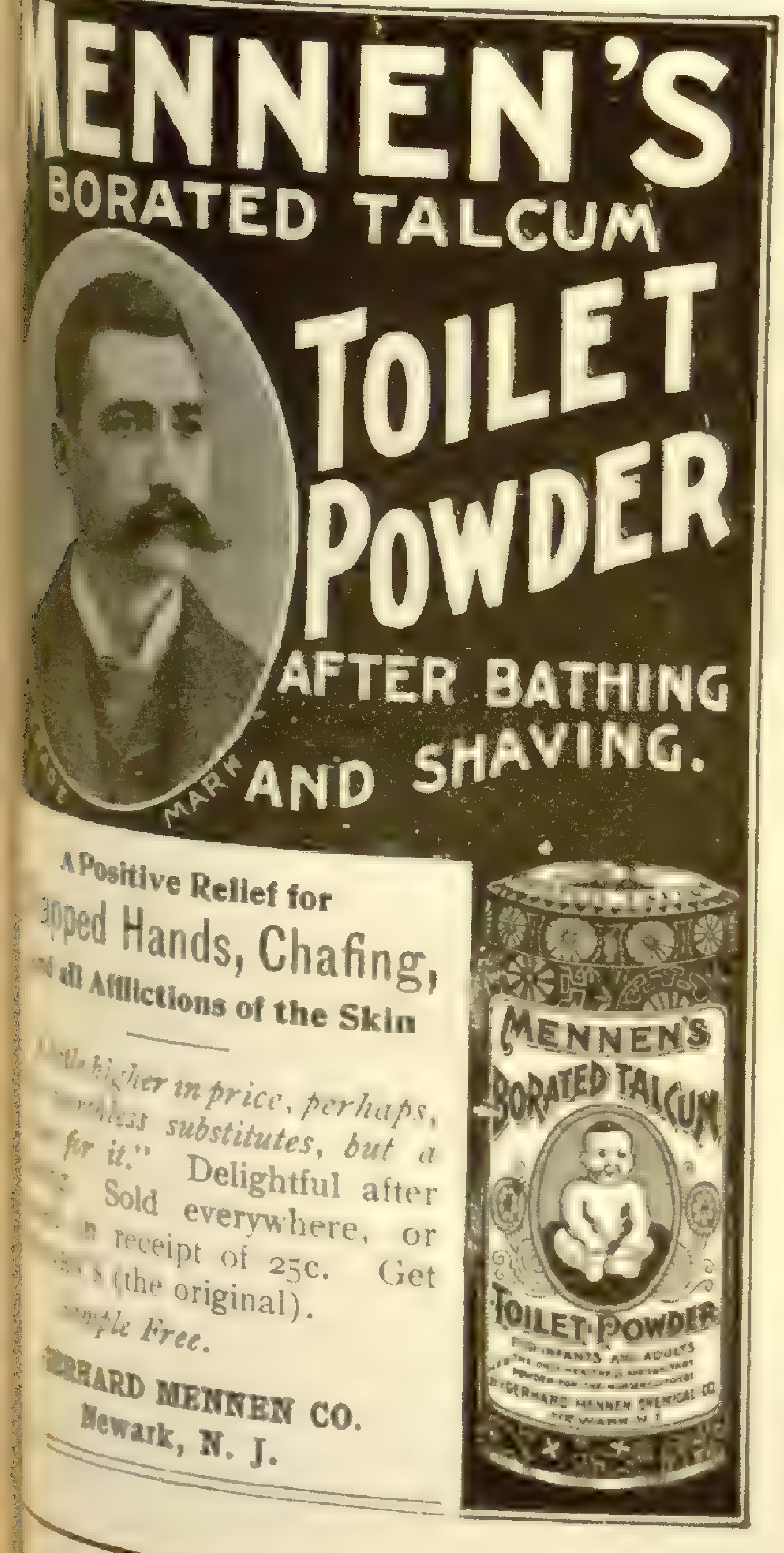

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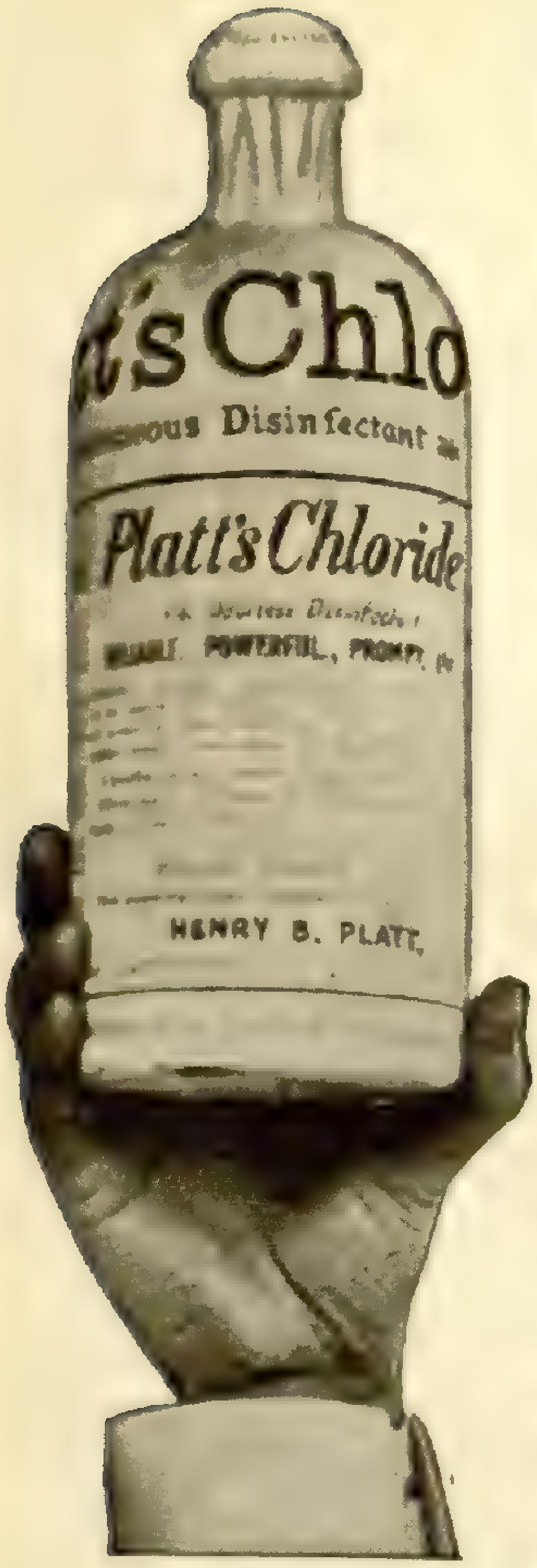

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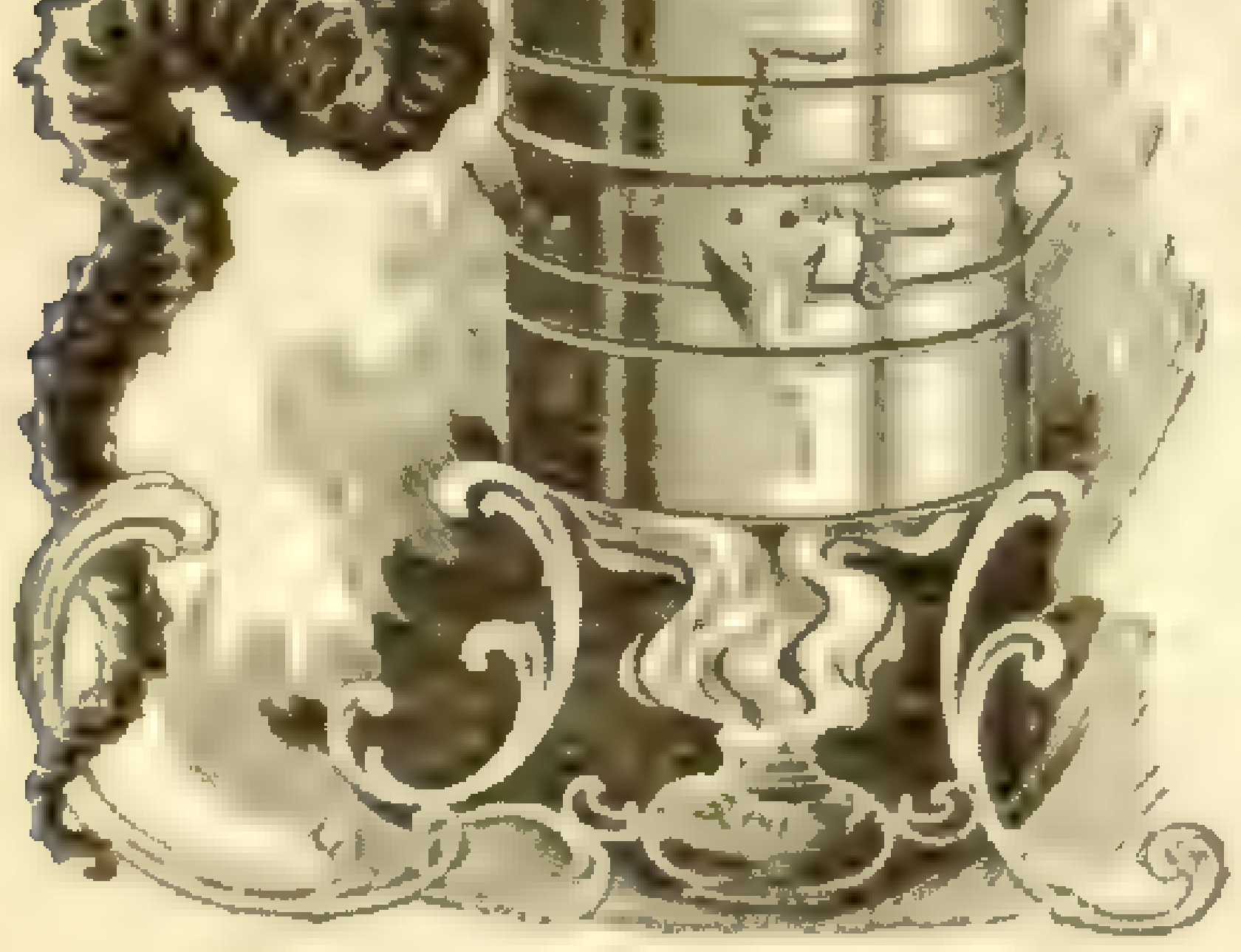

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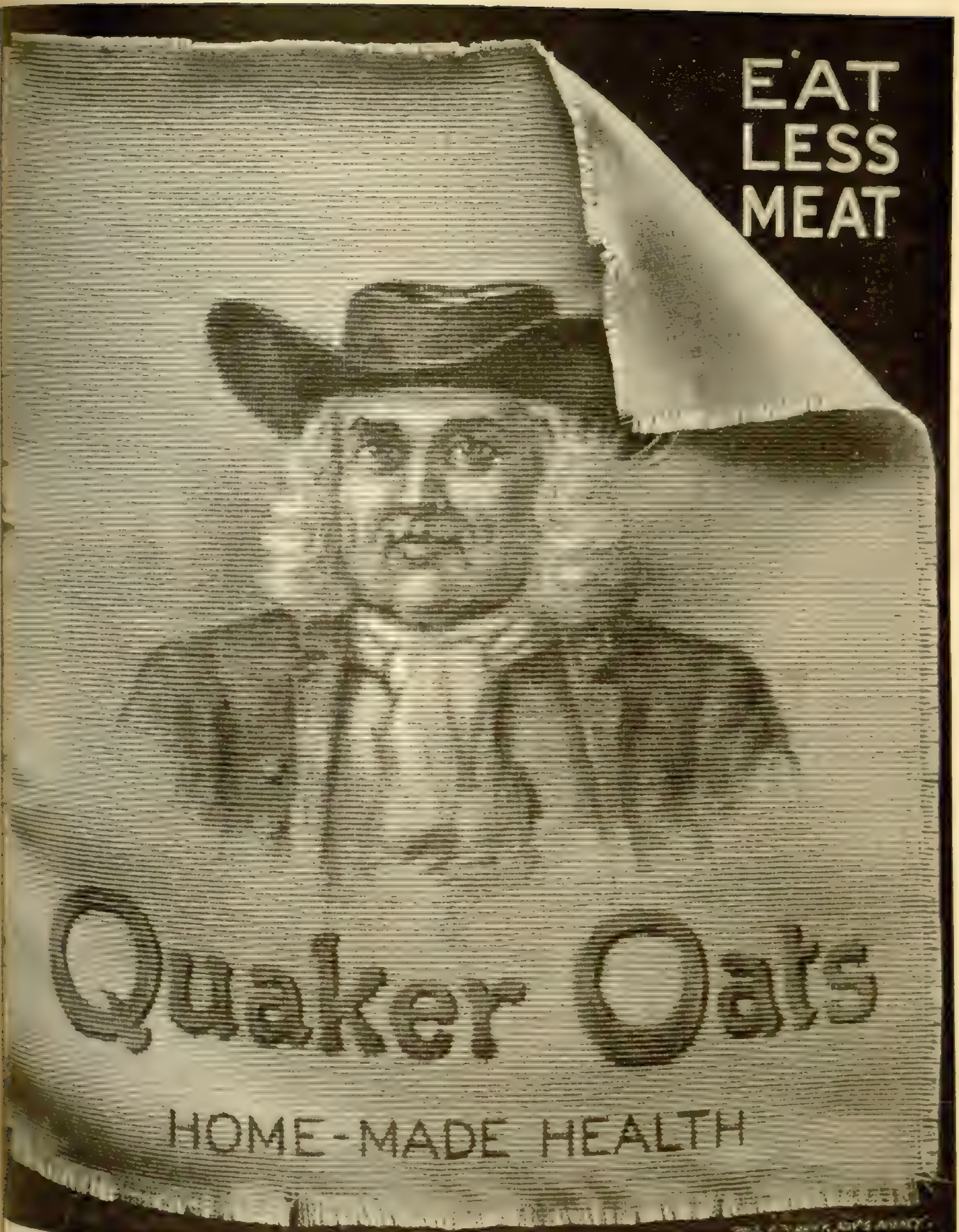

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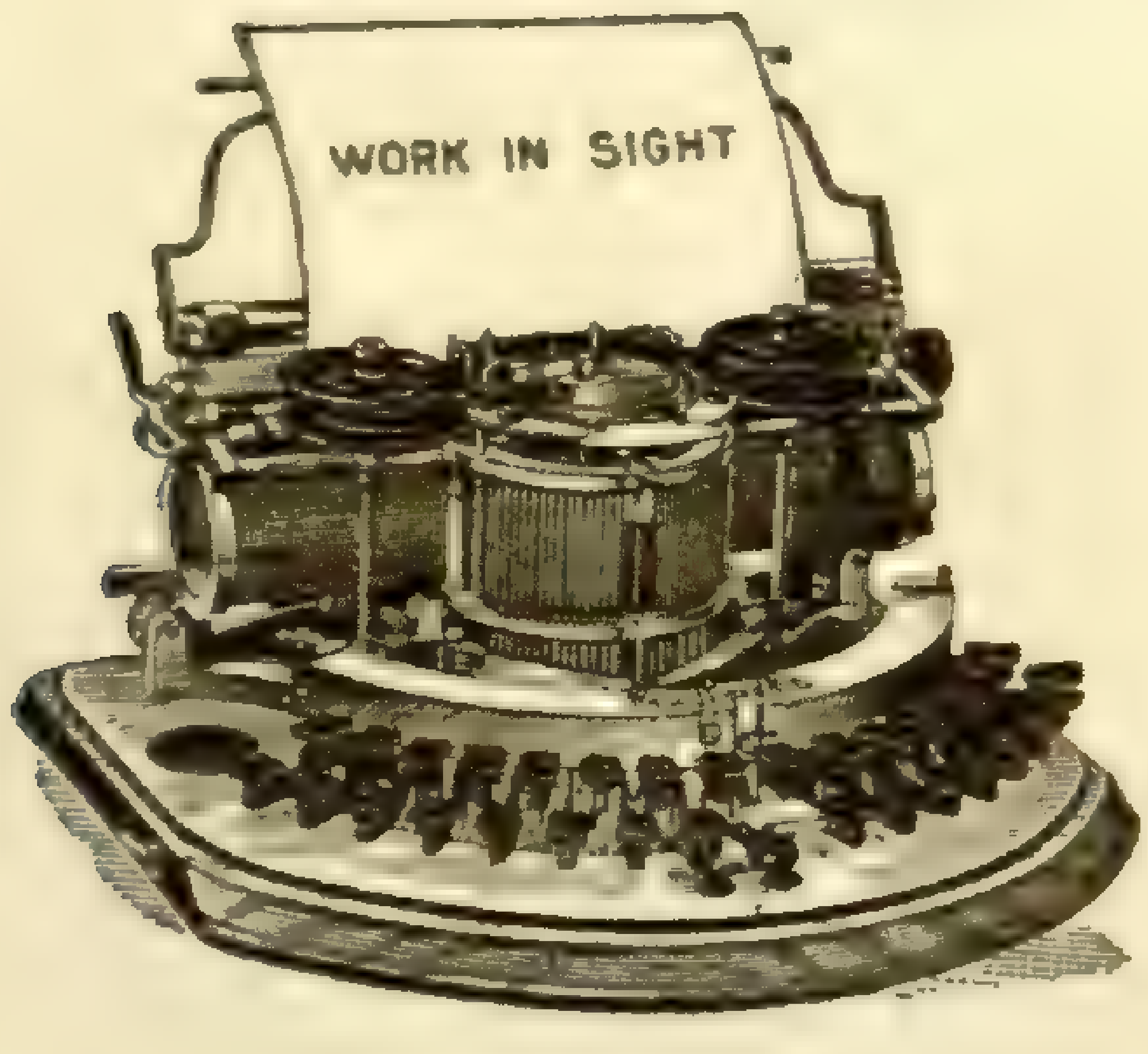

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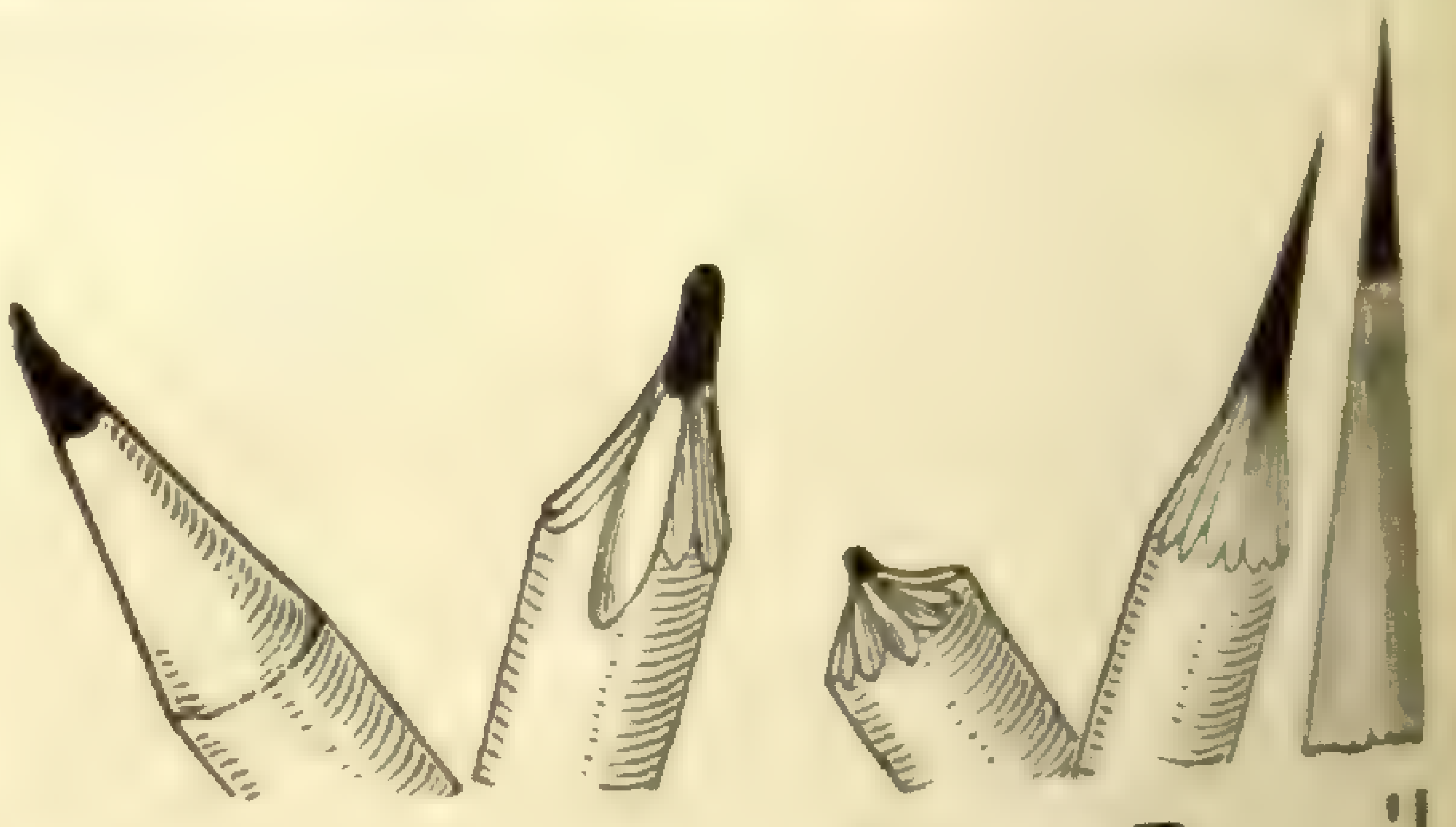

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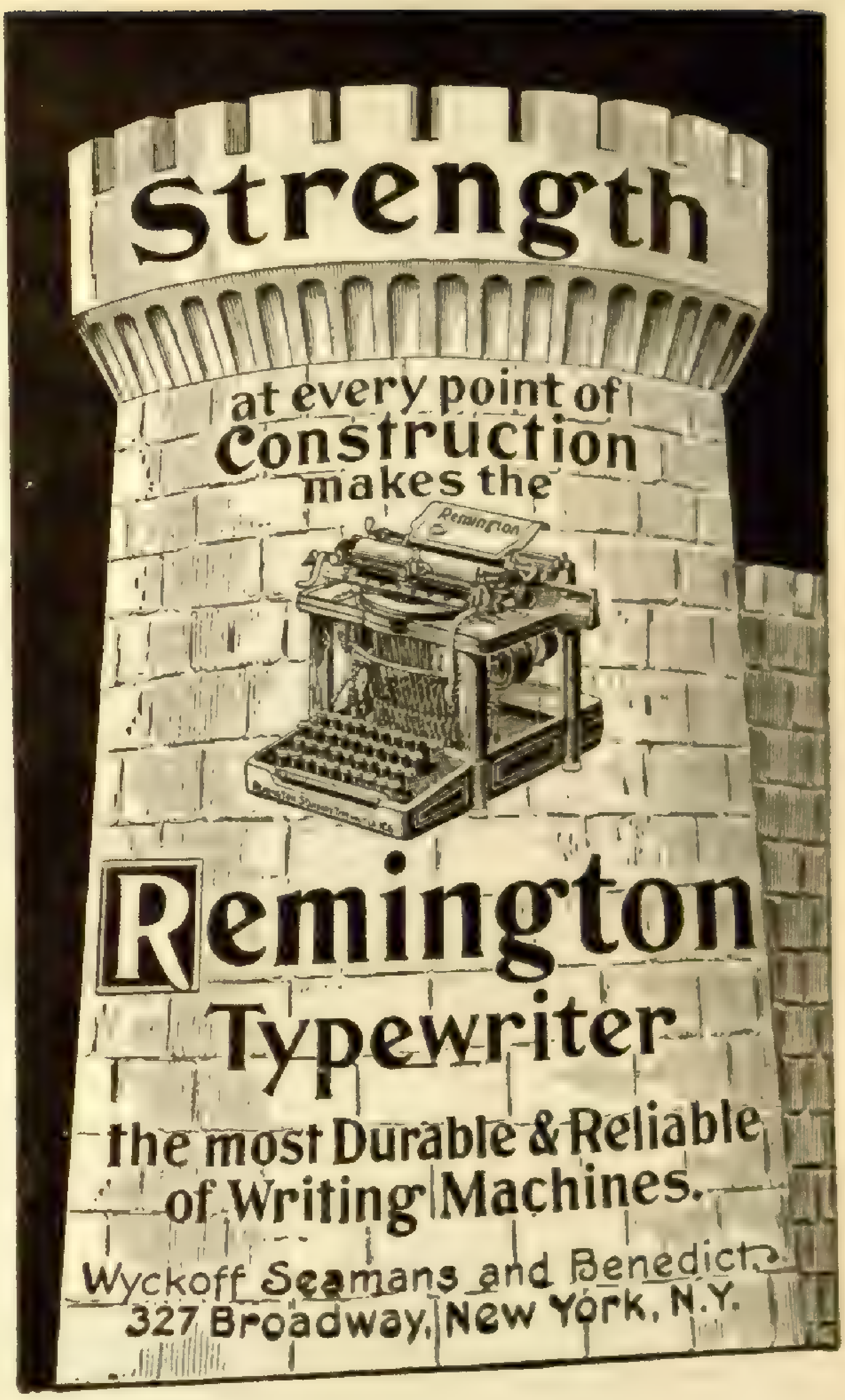
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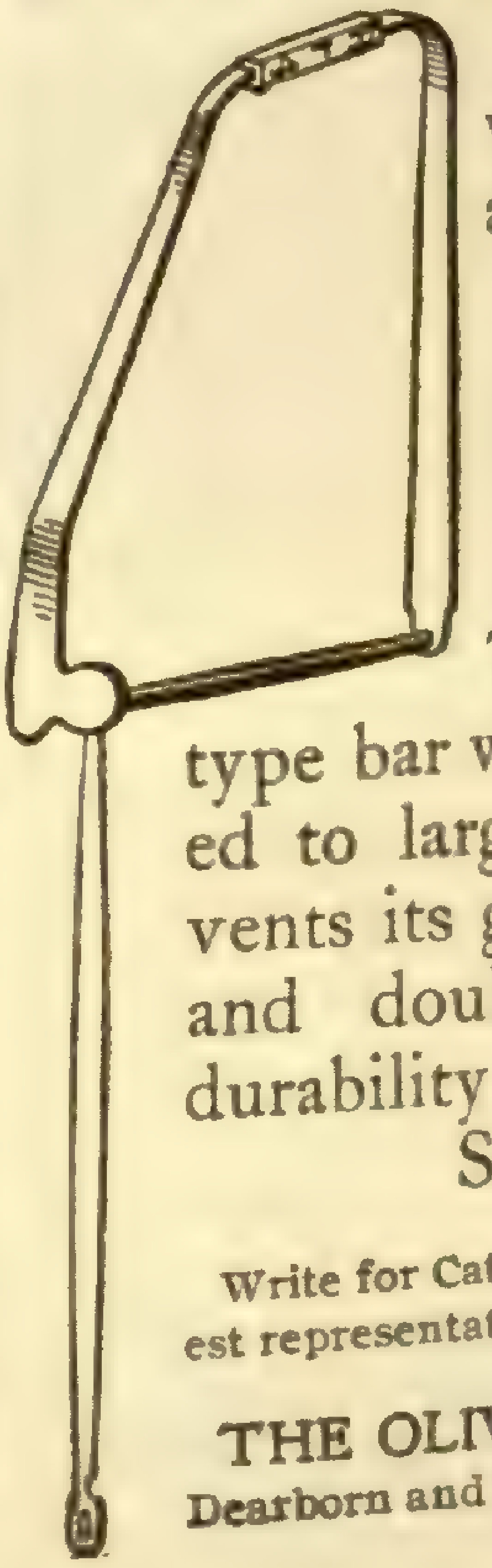

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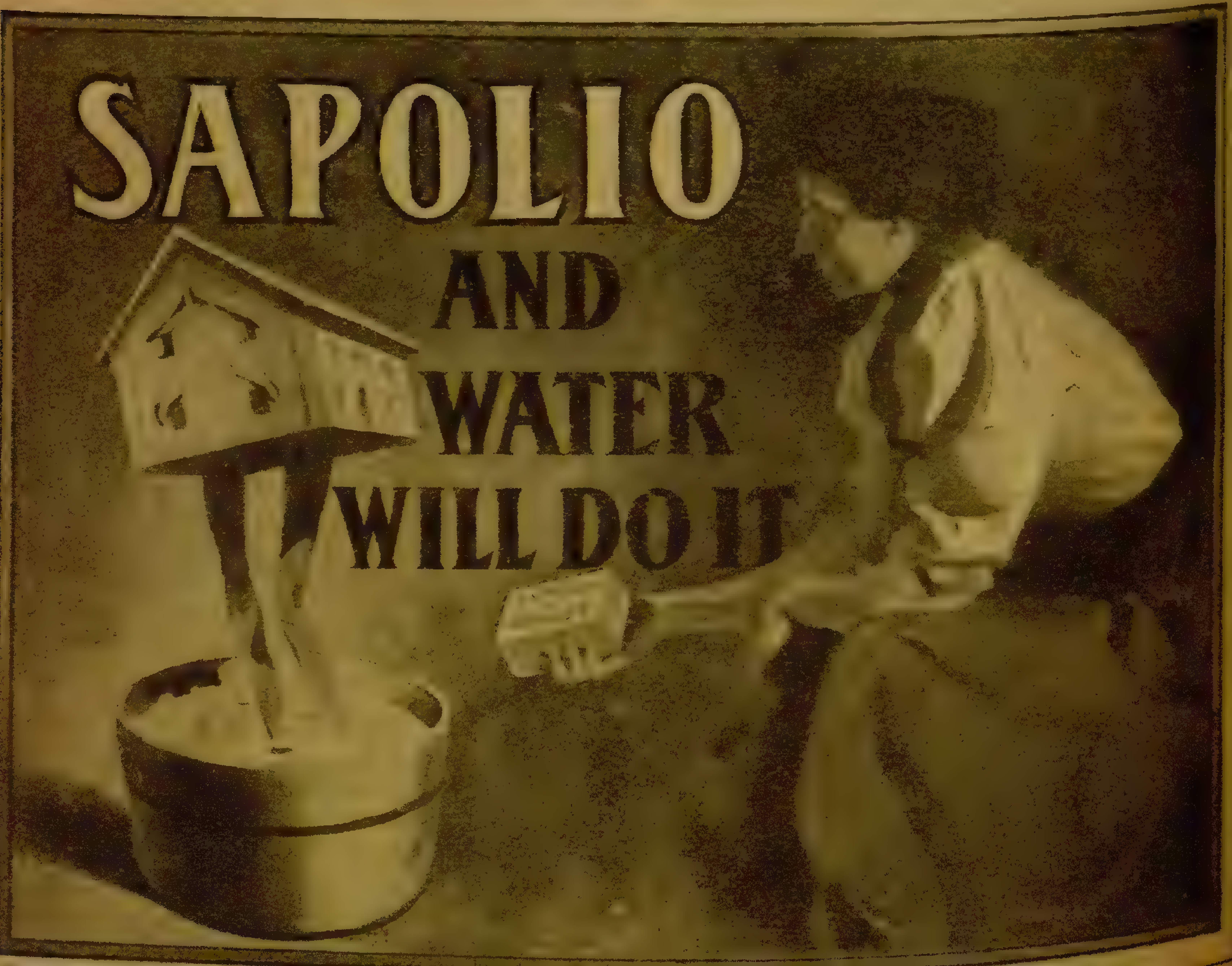

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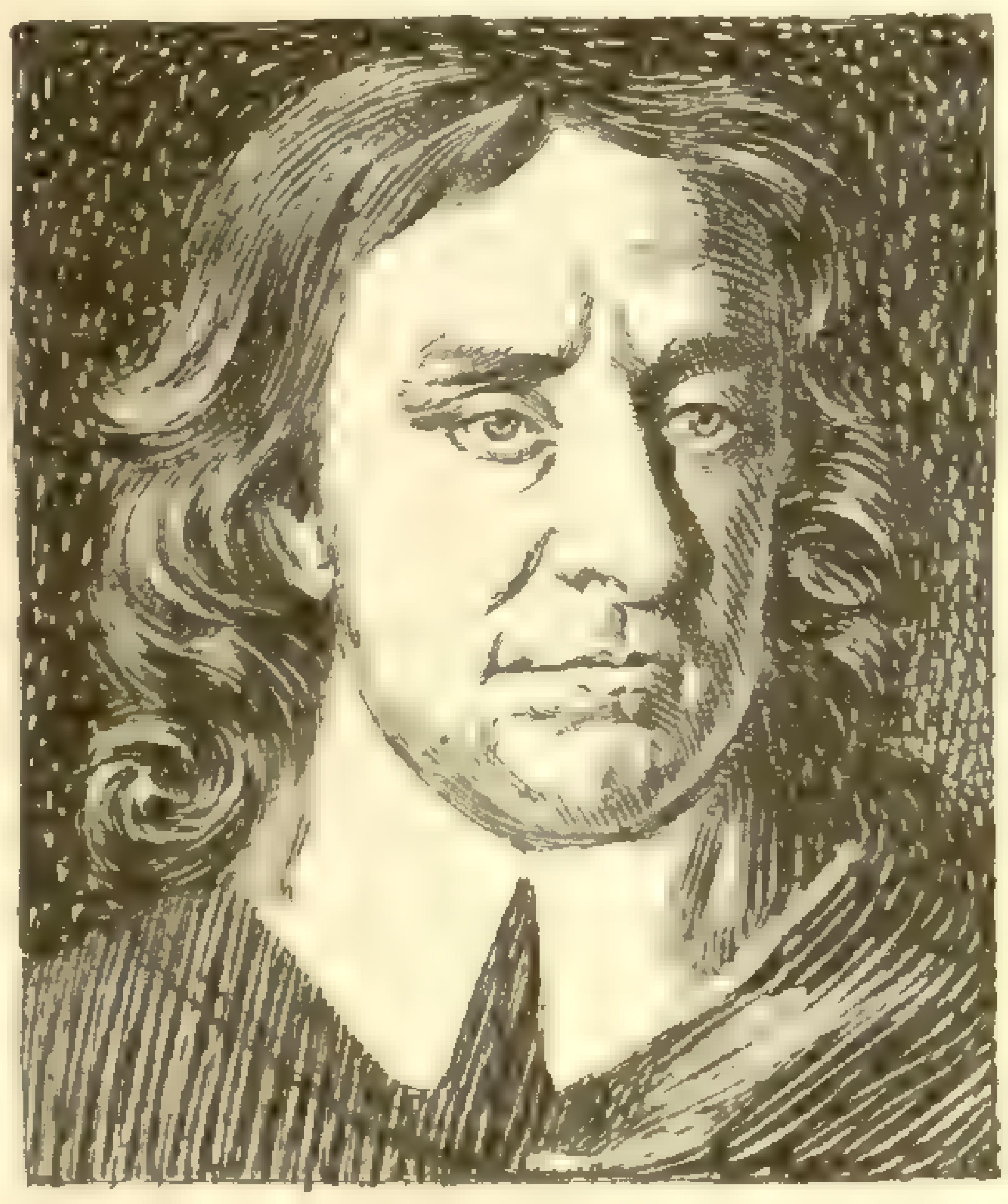

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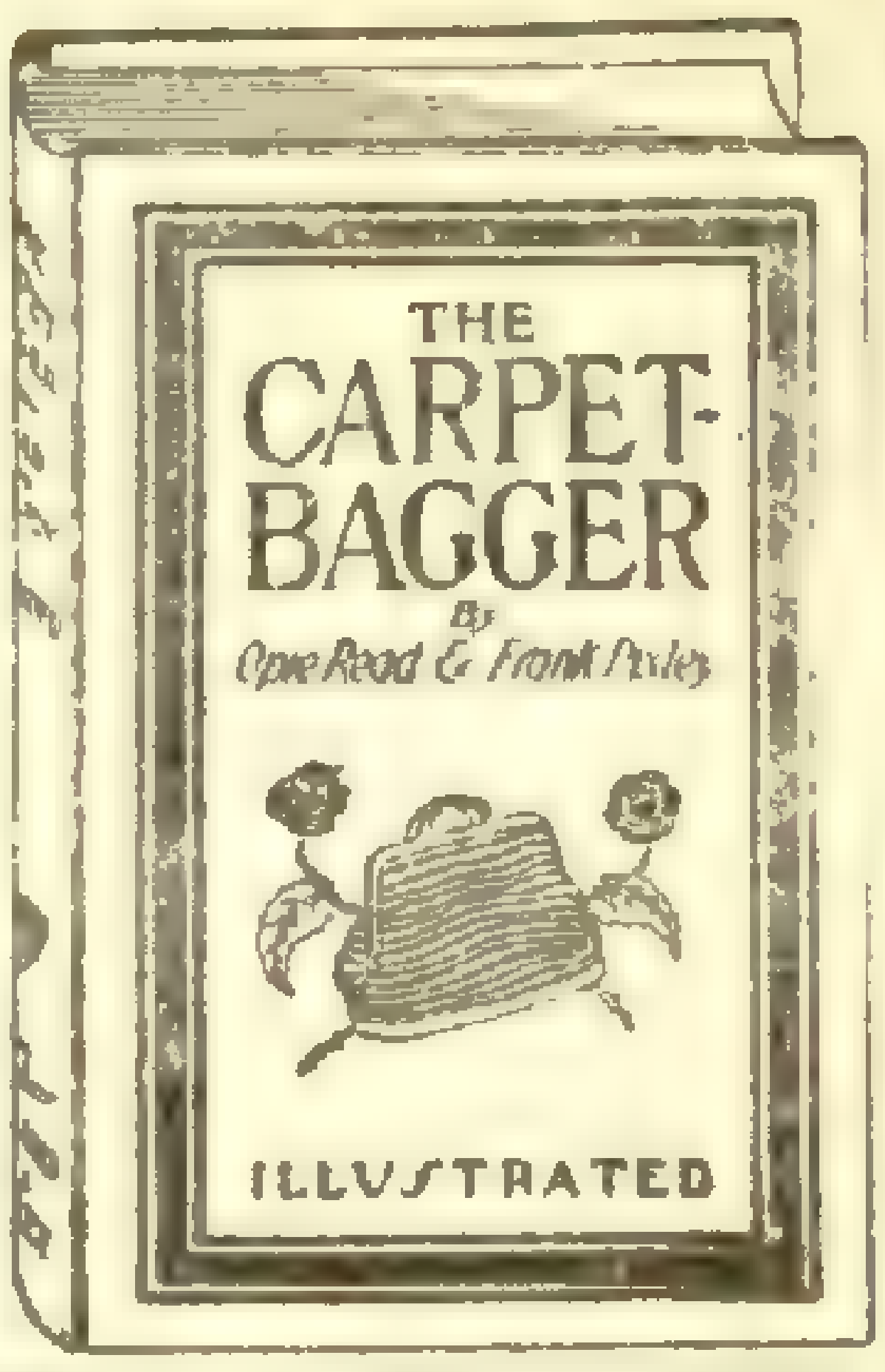
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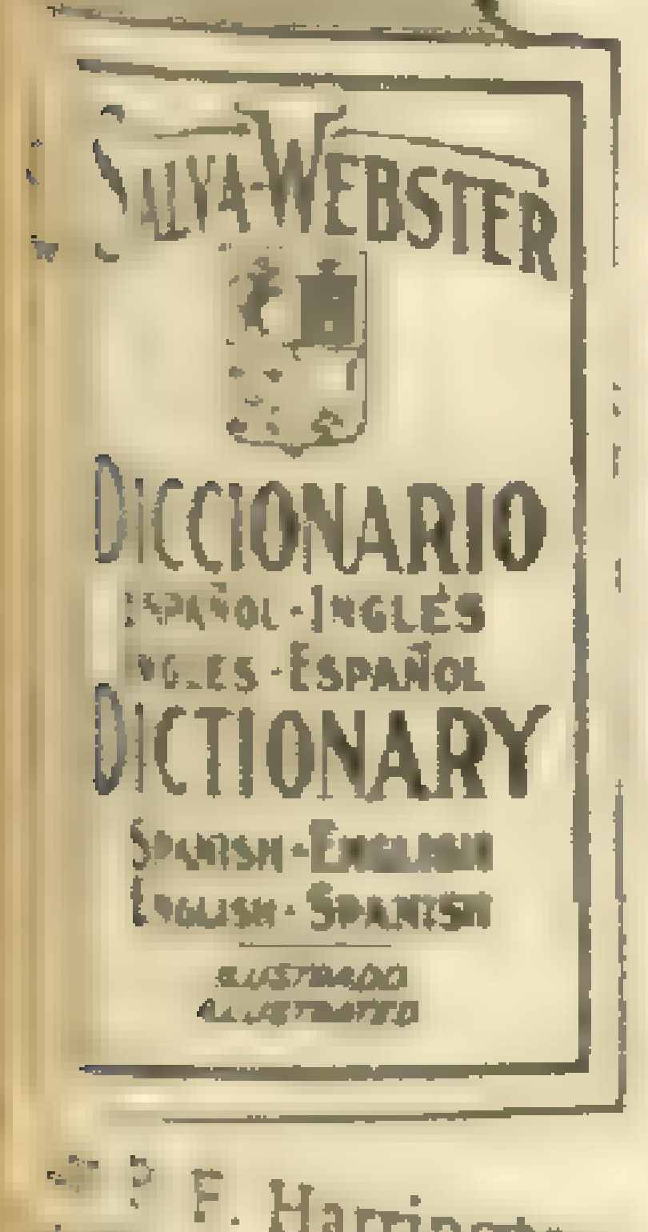
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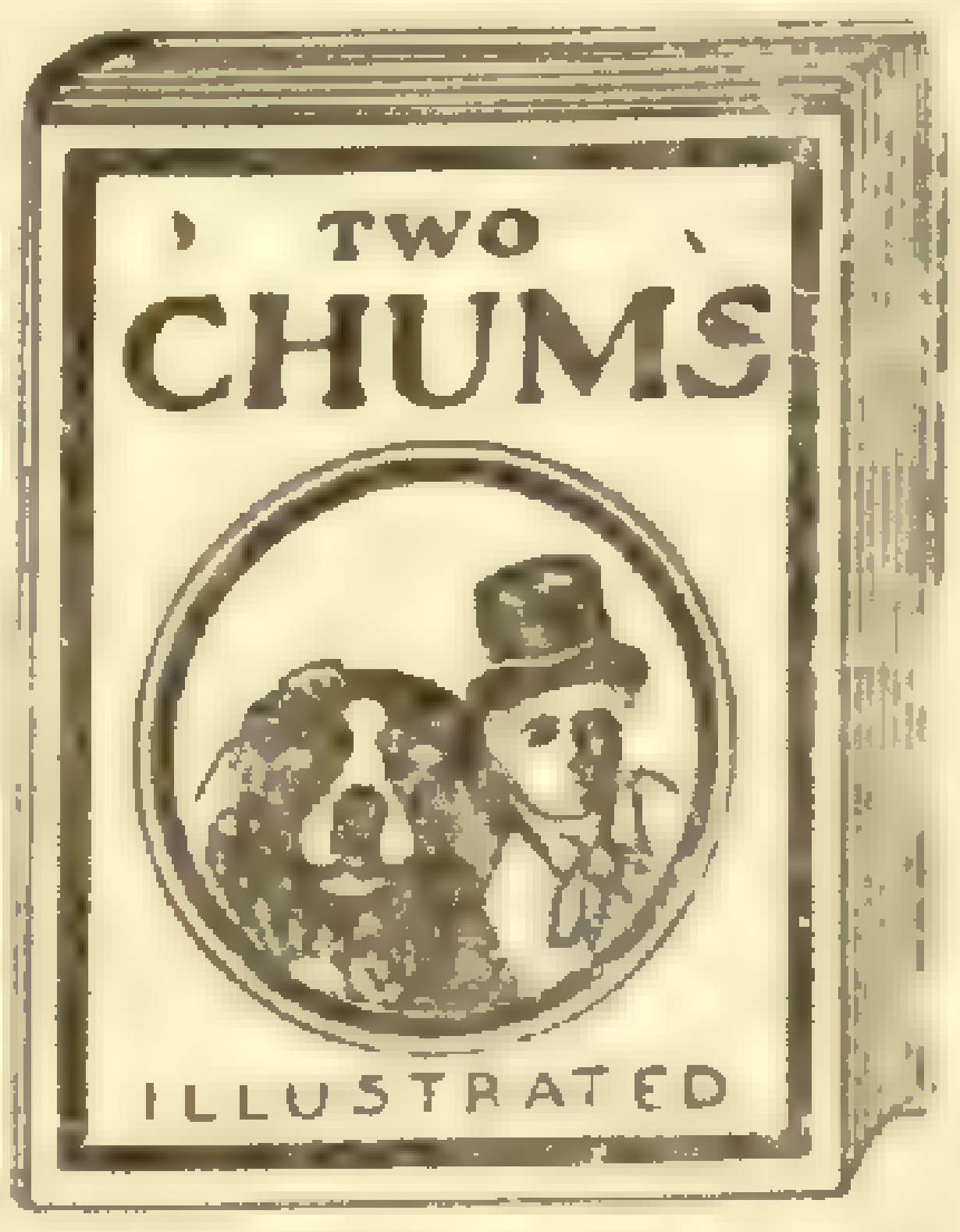
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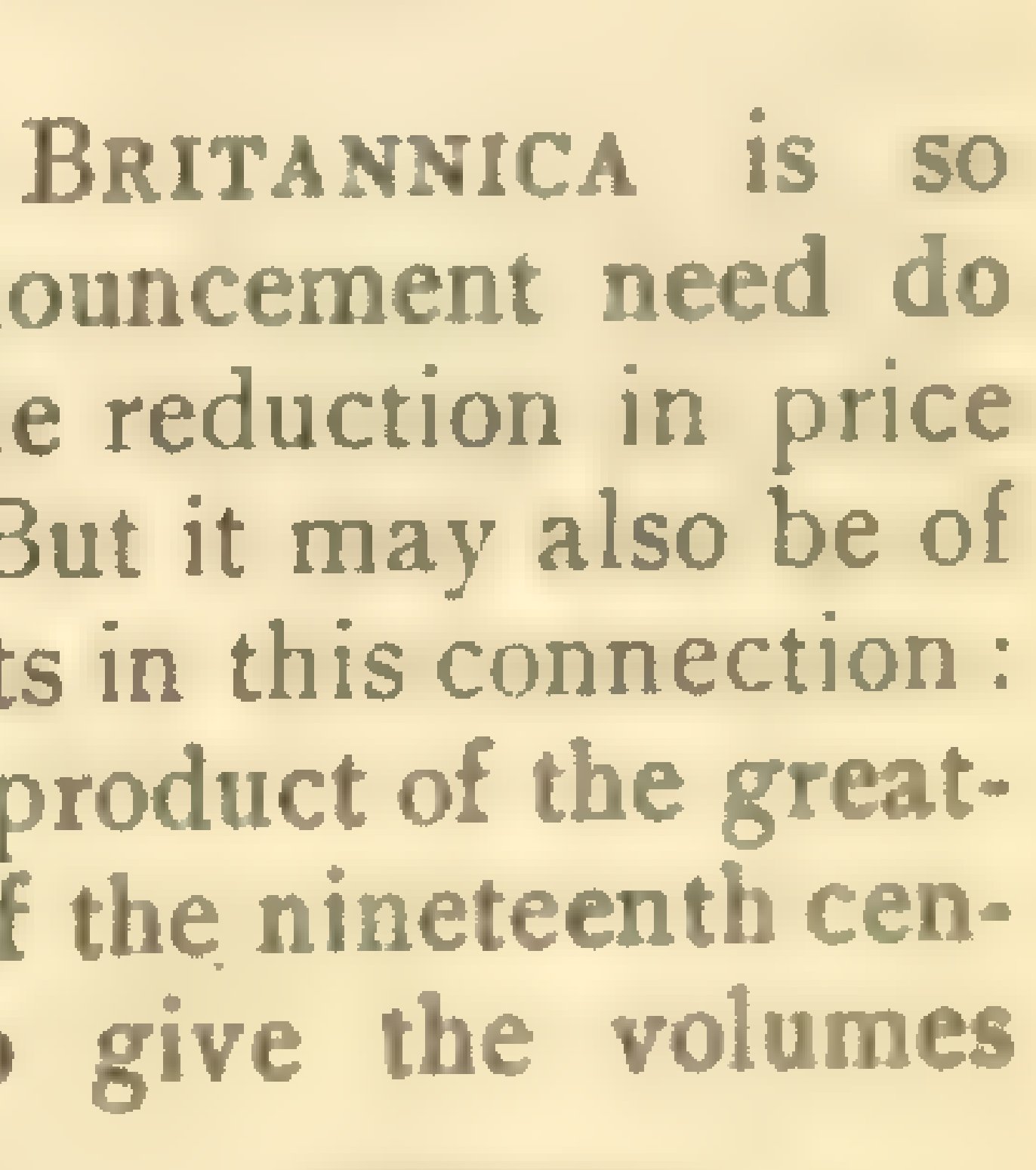
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VOLUME XXVIII NUMBER 6

BOTANICAL GAZETTE

DECEMBER 1899

SOME ROCKY MOUNTAIN CHRYSOTHAMNI.

AVEN NELSON.

AFTER an experience of several years as a collector and as a student of this group both in the field and in the herbarium, I have reached the conclusion that the genus Chrysothamnus, as it exists in the Rocky mountains, is still to an unsuspected degree unknown and unappreciated.

The plants of the far west became known to the botanical world at an earlier day than those of the interior west, and it seems to have been assumed that the species of the two regions ought to be the same. As a result of this assumption we have been producing a confusion in the herbaria that will be cleared up with difficulty. Dr. Greene's[1] papers on this genus represent the first attempt to do justice to the species of the range in question. In this genus, as in many others, Nuttall's knowledge obtained in the field has been discounted, but, like other attempts to subordinate his species, it has simply resulted in confusion.

The following notes it is hoped may be serviceable not only to resident students, but to all who are interested in this characteristic western genus. The new species that are proposed, while intended in some instances to simplify by segregation, are in others believed to represent species not heretofore collected. Only such species are included in this paper as belong to that natural botanical region of which Wyoming, with its high arid plains and mountain ranges, is the center. Localities mentioned are in Wyoming unless otherwise stated.

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[1] Erythea 3:92–96; 107–115. 1895.

CHRYSOTHAMNUS GRAVEOLENS Nutt.— For the literature of this species and a thoroughly usable characterization see *Erythea* **3**: 108. 1895. Its range as there given, and the habitat, "denudated soils," given by Nuttall, will prevent confusing with this certain other species that have been too often included. Thoroughly typical are the following collections: George E. Osterhout, New Windsor, Colo., September 8, 1898; the writer's nos. 503 and 2758 from the Platte cañon, in 1894 and 1896.

CHRYSOTHAMNUS PLATTENSIS Greene, Pitt. **4**: 42. 1899. *C. speciosus Plattensis* Greene, Erythea **3**: 111. 1895.—This is the representative in this range of the far northwestern *C. speciosus* Nutt.[2] I know of no specimens except from the eastern base of the Rockies. Well represented by the following collections: C. S. Crandall, Fort Collins, Colo., September 17, 1898; George E. Osterhout, New Windsor, Colo., September 8, 1898; E. L. Greene, LaSalle, Colo., September 10, 1896; the writer, Cheyenne, Wyo., August 27, 1896.

Chrysothamnus pulcherrimus, n. sp.—Shrub 0.5–1.5^{m} high, tree-like in form (a short trunk-like base much branched above); main stem and branches with grayish bark, the season's stems yellow but under the lens minutely lanate-puberulent, rather slender-virgate, terminating in an ample, compact, paniculate cluster: leaves moderately numerous, narrowly linear, rather lax and spreading, 5–8^{cm} long, from white-tomentose to greenish-glabrate: involucral bracts only two or three in each row, oblong, acute, nearly glabrous, ciliate on the margins: corolla tube longer than the throat into which it gradually expands, rather closely short-hairy: anthers well exserted, the appendages of the style exceeding these: akene softly pubescent.

A handsome shrub, its arboreous habit, numerous, slender branchlets, and large trusses of bright yellow flowers make it one of the strongly characterized and conspicuous species. It is to be distinguished from *C. graveolens*

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[2] This and its var. *albicaulis* is sometimes reported from this range, but I believe cannot be authenticated.

Nutt., to which it is allied on the one side, by its less glabrate condition, its narrower leaves, yellow stems, paniculate (not corymbose) inflorescence, and proportionately shorter style appendages. From *C. speciosus* Nutt., to which it must be compared on the other side, by its stout yellow (not whitish) branchlets, its longer less filiform leaves, glabrate involucral bracts, pubescent corolla tube, and more open-paniculate inflorescence.

Under no. 2066 it has been distributed as *C. speciosus*, from Cummins, August 10, 1896. Collected near the same locality (Wood's Landing), August 9, 1897, no. 3477, the latter taken as the type number. Specimens from Gros Ventre river, August 23, 1894, no. 966, is a form with more virgate and glabrate branchlets.

CHRYSOTHAMNUS PULCHERRIMUS **fasciculatus,** n. var.— size and habit of the species; the season's branchlets short, numerous, forming a brush-like fascicle at the ends of the woody branches: leaves numerous, short ($2–3^{cm}$), somewhat rigid, green or yellowish-green, with a thin tomentum: inflorescence similar to that of the species but smaller, terminating the numerous branchlets.

The fascicled branchlets and short rigid leaves suggest *C. collinus* Greene, but the size, habit, inflorescence, and floral characters are those of *C. pulcherrimus*. *C. collinus*, in its pubescent long acuminate bracts, is quite in contrast to this.

First collected in 1894 on Boulder creek, August 26, no. 1120; in 1897 at Creston, August 28, no. 4419 and distributed as *C. speciosus.*

CHRYSOTHAMNUS FRIGIDUS Greene, l. c.—What is typical of this species is not quite clear (see note under var. *concolor*), but it seems plain that it was intended to include that superabundant but variable species of the high plains, popularly known as "rabbit-brush." It is illustrated by Dr. Greene's specimens from Laramie, August 10, 1895; the writer's nos. 2787, 5280, 5282, 5315.

CHRYSOTHAMNUS FRIGIDUS **concolor,** n. var.—Yellowish-green throughout except for the bright yellow flowers, $3–4^{dm}$ high from a barely shrubby base, the numerous stems rather slenderly virgate, terminating in a narrow thyrsus (sometimes paniculate corymbose): leaves linear, erect or irregularly spreading, $4–7^{cm}$ long, $1–2^{mm}$ wide: bracts mostly acute, the outer with a light tomentum like that of the stems and leaves, all with a thin, scarious-ciliate margin: corolla tube short-pubescent.

Though I believe this to be a species, I hesitate to separate it from *C. frigidus* so long as I am unable to say what is typical of the latter. Ever since its publication (*Erythea* 3 : 112. 1895), based upon specimens (one of which I have) collected at Laramie by Dr. Greene, I have collected freely in this genus. Though in the center of distribution for *C. frigidus*, and in spite of the large series representing it, I have never secured duplicates of the specimens distributed by Dr. Greene, nor any quite typical, judged by the description. Assuming that the plant, so common on the Laramie plains, of which there seem to be several forms, must stand as *C. frigidus*, the preceding may at least be ranked as a variety, no matter what may be typical of the other. Nor may this be connected with *C. Plattensis*. Some forms of that seem to connect very closely with *C. frigidus*, but typical specimens of the two are really widely different. *C. Plattensis* one at once associates with *C. speciosus* on general appearance; not so with *C. frigidus* and the variety now proposed.

Collected on the banks of Hutton lakes, in rather sandy, but more or less alkali impregnated soil, September 6, 1898, no. 5300.

Chrysothamnus pallidus, n. sp.—A small tufted shrub, 2–5^{dm} high, with a close felted tomentum which persists even on the old stems: stems rather scraggy branched, somewhat rigid, the season's twigs very short: leaves mostly confined to the season's growth, the tomentum looser than on the stems, ascending, irregularly spreading or reflexed, linear, acute, 2–3^{cm} long, shorter and crowded at the base of the inflorescence: heads small, in thyrsoid panicles or somewhat corymbosely clustered cymes; involucre short, sub-campanulate, its bracts short, oblong, subacute, ciliate-pubescent especially on the margins, about three in each row: tube of the corolla closely covered with short, clavellate hairs, the throat longer than the tube and cleft one third its length: style divisions exserted, the stigmatic portion about as long as the appendages: akenes densely pubescent, about equaling the corolla tube.

Allied to *C. frigidus* but distinguished by its more rigid, scraggy branches which are leafy only on the branchlets; by the close, persistent, white indument; by the shorter bracts, florets, akenes; by the exserted style divisions and the denser hairiness of the corolla tube.

Seemingly a rare plant; collected on an alkaline flat near Laramie, September 24, 1898, no. 5347. Also on Bacon creek, August 15, 1894, no. 910.

Chrysothamnus Wyomingensis, n. sp.—Tufted, 2–4^{dm} high, bushy-branched from the base, the branches with ascending or erect, yellowish-green branchlets, with a thin inconspicuous tomentum throughout: leaves rather numerous, especially above, 4–6$^{\rm cm}$ long, narrowly linear, sharp-pointed, plane or somewhat canaliculate, viscidulous as are also the branchlets: inflorescence a narrow thyrsiform-panicle, rather leafy, at maturity barely surpassing the uppermost leaves: heads about 12$^{\rm mm}$ high; bracts few (10–14), not in strict vertical ranks, mostly acute or acutish, glandular on the greenish keel, from glabrate to ciliate-pubescent: corolla tube and throat scarcely distinguishable, but slightly expanded upward, the lobes about one eighth of the whole length, obscurely short-pubescent below: style branches exserted, the appendages longer than the stigmatic portion.

This species has been secured but twice, both times on strongly saline soil, viz., at Buffalo, July 25, 1896, no. 2495, and on Vermilion creek, July 24, 1897, no. 3590. Its nearest ally in habit and some other characteristics is *C. frigidus*, but in form of inflorescence it approaches *C. Parryi* (Gray) Greene. It differs from *C. frigidus* in being almost devoid of tomentum, in its yellowish branches, its green leaves, narrow leafy inflorescence, and its exserted styles. It is also an earlier plant, one of the earliest of the several species of this genus in this region.

CHRYSOTHAMNUS PARRYI (Gray) Greene, l. c.—The habitat and range of this well-known species is always given as "parks of the Rocky mountains in Colorado," but certainly similar parks in southern Wyoming, at least, must be added. Typical specimens are, J. H. Cowen, Breckinridge, Colo., August 1896; the writer's nos. 2617 and 3495, Lincoln gulch, August 1896 and Big creek, August 1897.

CHRYSOTHAMNUS HOWARDI (Parry) Greene,[3] l. c.—The habitat of this, like the preceding, is given as "parks of the Rocky mountains," but it should be stated that the *parks* are very different in their character. *C. Parryi* inhabits moist open ground, known as parks, occurring at intervals in the timbered mountain ranges. *C. Howardi* inhabits that other class of parks, viz., extensive, high, dry table-lands like North Park, Colo. and the Laramie plains. It occupies the dry foothills and ridges and I doubt not may occur on the eastern base of the Rockies, possibly extending into eastern Nebraska, as given in Britton & Brown's *Flora*, a statement questioned by Dr. Greene. It seems probable that some confusion on this point has arisen by the distribution of a somewhat similar plant occurring in situations much like those in which *C. Parryi* occurs. This plant I think should stand as a species, and may be named as below.

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[3] Dr. Greene's papers cite the literature of all the well-known species so fully that it here seems necessary only to call attention to this fact.

Chrysothamnus affinis, n. sp.—Scarcely shrubby, the persistent base hardly more than a much-branched woody caudex: the season's stems very numerous, simple, 1–2^{dm} high, yellowish, glabrate: leaves crowded, narrowly linear, acute, erect or spreading, dark green, nearly glabrous, 3–4^{cm} long: inflorescence a crowded spicate thyrsus which at maturity distinctly surpasses the leaves: bracts glabrate, arachnoid-ciliate on the margins, somewhat thickened-coriaceous, about three in each row; the outer with an ovate base, contracted in a usually spreading acumination; the inner linear-oblong, abruptly acuminate, shorter than the pappus: corolla tube slender, bearing only a few, minute, scattering clavellate hairs, shorter than the expanded, tubular throat which is cleft about one fourth its length: style appendages tardily but at length wholly exserted: akene linear-cylindric, about 6^{mm} long.

Allied to *C. Howardi* (Parry) Greene, but clearly distinct by its less shrubby habit, its greenish glabrate aspect, and its crowded yellow inflorescence which surpasses the leaves. *C. Howardi* has cinerous leaves and stems, a dirty whitish-yellow inflorescence, and the leaves overtop the comparatively few and large heads whose bracts are distinctly arachnoid.

Excellent specimens were collected by J. H. Cowen, Jefferson, Colo., August 1896, and distributed as *Bigelovia Howardi* Gray. Type in Herb. University of Wyoming.

CHRYSOTHAMNUS AFFINIS **attenuatus,** n. comb. (*Bigelovia Howardi attenuata* Jones, Proc. Cal. Acad. Sci. II. **5**: 691. 1895.—The specimen of Mr. Jones' type number, 5912, seems to approach *C. affinis* much more closely than it does *C. Howardi*. Its more persistent stems and longer branchlets, its very long acuminate bracts, and long exserted styles will readily distinguish it.

CHRYSOTHAMNUS COLLINUS Greene, Pitt. **3**: 24. 1896.—This distinct and clearly characterized species I think has not been collected except by the writer (no. 4435, Point of rocks, August 1897, near the type locality) since the original specimens were collected at Rock springs by Dr. Greene.

Chrysothamnus oreophilus, n. sp.—Green and nearly glabrous, 2–4^{dm} high: stems several to many, from a thick woody base, strictly erect and somewhat fascicled, grayish with a thin tomentum, the annual twigs arising from near their summit, these also fascicled-erect, slender and yellowish-green: leaves erect, linear-filiform, very acute, canaliculate, green and glabrate, 3–5^{cm} long: heads small (about 1^{cm} high), in small fastigiate cymes: bracts oblong, abruptly sub-acute, only two or three in each vertical row, the scarious margins ciliate-pubescent: corolla sparsely short-hairy, divisible into three equal regions (tube, throat proper, and a transition region); lobes more than half as long as the tube proper, distinctly glandular-thickened at apex: pappus rather sparse: style appendages longer than the stigmatic portion, at length exserted: the short akene finely pubescent.

The erect habit of stems, the twigs and leaves, the greenish aspect, and numerous but small flower clusters mark this as peculiarly distinct from the other species of this range. It is abundant on stony slopes in the Bear river hills, near Evanston. Type number 4105, July 27, 1897. Represented also by M. E. Jones' no. 6040, distributed unnamed.

CHRYSOTHAMNUS PUMILUS Nutt., Trans. Am. Phil. Soc. **7**: 323. 1840.—The recharacterization by Dr. Greene,[4] after every possible effort has been made to settle what was the original of the species, is of noticeable service to us all. This common and somewhat variable species is now recognizable. It is well represented by the following numbers from various parts of Wyoming: 617, 903, 1121, 1197, 2883, 3524 and 5398. The last, from Hutton's lake, September 7, 1898, is typical so far as I am able to judge.

CHRYSOTHAMNUS PUMILUS **varus**, n. var.—Smaller than the species, only 1–3^{dm} high, the shrubby base divaricately scraggy branched, the season's branchlets slender, very numerous, 5–15^{cm} long, with a whitish or straw-colored bark: leaves glabrous except for an obscurely scabrous margin, linear, almost filiform, one-nerved, somewhat involute and usually more or less twisted, irregularly and widely divaricate, very numerous on the new wood and often fascicled on two-year old branches, short, rarely exceeding 3^{cm}, usually much shorter: inflorescence and bracts much as in the species.

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[4] Erythea **3**: 93. 1895.

This is what has often been called in this range *Bigelovia Douglasii stenophylla* Gray. I have distributed some specimens under that name, but I am now satisfied that that is a very different plant and belongs to a more western range.[5] It occurs mostly on dry ridges and stony or sandy slopes. Type of the variety is no. 1847, Centennial valley, August 26, 1895. Another collection is no. 4434.

CHRYSOTHAMNUS PUMILUS **acuminatus**, n. var.—The habit of the species: numerous slender stems from a woody base $2-3^{dm}$ high, with whitish bark: leaves numerous but early deciduous below, crowded toward the inflorescence, nearly filiform, ascending, somewhat twisted, $2-3^{cm}$ long: inflorescence more paniculate than in the species; bracts lanceolate, long-acuminate with more or less spreading tips, nearly equaling the 5–6 flowered disk.

This variety I have from La Veta, Colorado, only, collected by Professor C. S. Crandall, August 21, 1897.

CHRYSOTHAMNUS LANCEOLATUS Nutt. l. c.—I see no reason for reducing this to a variety. Numerous collections of it show as much constancy in the essential characters as most of the recognized species. Its low tufted habit, its uniformly scabro-puberulent surface, and its either plane or twisted lanceolate leaves make it not hard to recognize. Specimens by the writer, nos. 889, 905, 2672, 2793, 5294 and 5314 are representative, as is also Professor Crandall's from Walden, Colorado, July 1894.

CHRYSOTHAMNUS GLAUCUS Aven Nelson, Bull. Torr. Bot. Club **25**: 377. 1898.—At the time this was published only meager material was at hand, but it has since been secured in abundance. The characters as given are well borne out, except that in old specimens the leaves are less glaucous.

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[5] I think it should be noted that *C. viscidiflorus* Nutt. l.c. (*Bigelovia Douglasii* Gray) occurs probably in a range to the northwest of that now under consideration, and that several of its varieties, though often attributed to the eastern Rockies, are not in this range at all. Among such may be named vars. *latifolius*, *serrulatus*, and *tortifolius*.

CHRYSOTHAMNUS LINIFOLIUS Greene, Pitt. **3**: 24. 1896.—This I think has not been secured except in south-central Wyoming, where it occurs on the banks of strongly saline creeks. Its halophytic and hydrophilous nature is very marked, as it often grows with its "feet" in water so strongly impregnated with salts as to be wholly unfit for any use whatever. Its original collection was by the writer, on Poison Spider creek, no. 618, July 1894, but it was published upon material collected by the author of the species at Rock springs, August 1896. The writer has collected it also at Bitter creek and at Granger, nos. 4143 and 4137.

CHRYSOTHAMNUS VASEYI (Gray) Greene, Erythea **3**: 96. 1895.—As pointed out by Dr. Greene, this has an akene quite at variance with the species that have preceded. The range given for it is too limited, for certainly very characteristic specimens are at hand as follows: G. E. Osterhout, North Park, Colorado, September 1897; by the writer, Big creek, August 11, 1897, no. 3494, and near Laramie, September 1898, no. 5331.

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ON THE TOXIC EFFECT OF DELETERIOUS AGENTS ON THE GERMINATION AND DEVELOPMENT OF CERTAIN FILAMENTOUS FUNGI.

J. F. CLARK.

(*Concluded from p. 327.*)

ALL acids tested retard germination and early mycelial development of the mold fungi. In the case of the mineral acids this retarding action is usually evident in $\frac{n}{1024}$ concentration. The acetic acids do not have any perceptible influence at this dilution. Cultures which are only slightly retarded almost invariably take on new vigor a few hours after germination, and overtake and surpass the checks in development of mycelium. This excessive mycelial development is usually accompanied by retardation of fruiting, and usually reaches its maximum in cultures two or three removes from the inhibiting concentration. Cultures in the acetic acids show a greater stimulation of mycelial development than in HCl or H_2SO_4. Cultures in HNO_3 resembled those in the acetic acids in development of mycelium but were not so greatly retarded in fruiting.

Smaller quantities of acid on an average proved injurious to Œdocephalum than to the other forms, $\frac{20n}{2048}$ being on the average distinctly detrimental. In this respect the others came in the following order: Botrytis, 23; Penicillium, 28; Aspergillus, 42; Sterigmatocystis, 64.

Œdocephalum was also the most easily inhibited, its relative inhibiting coefficient being 72; followed by Botrytis, 76; Penicillium, 100; Aspergillus, 104; and Sterigmatocystis, 200.

Botrytis, however, was the most easily killed. The order in this respect was: Botrytis, 100; Œdocephalum, 137; Aspergillus, 272; Sterigmatocystis, 369; and finally Penicillium, whose spores showed by far the greatest resistance, represented by the coefficient 498. This high resistance of Penicillium in regard to the death-point may be partly due to the tendency of the spores to adhere in bunches in making the inoculation, a difficulty never fully overcome.

In table II a comparison of the chemical affinities of the different acids tested by seven methods is given.

TABLE II.

CHEMICAL AFFINITIES OF ACIDS.

HCN	Tri.	Di.	Mono.	Acetic	$\frac{1}{2}H_2SO_4$	HNO_3	HCl	Acid
—	62.3	25.3	4.9	1.4	65.1	99.6	100	Relative ionization in $\frac{n}{1}$ sol.
—	68	23	4.3	.34	74	92	100	Catalysis of methyl acetate
—	63	18	5.1	1	67	110	100	Catalysis of calcium oxalate
—	82	34	7.2	1.2	68	102	100	Neutralizing hydroxids
—	75.4	27	4.9	.4	73	100	100	Invertion of cane sugar
—	96.7	62.4	17.2	4.7	71.9	99	100	Multirotations of dextrose
200+	200	100+	100	25	100	100	100	Physiological action on Lupinus
7666	255	359	396	277	112	163	100	Ditto, on molds

Line 1 gives the relative ionization of these acids (except HCN) in normal solution. HCl being the basis of comparison here as in the succeeding tests is given 100 units. Line 2 gives the relative powers of the different acids to promote the well known catalysis of methyl acetate in aqueous solution. Line 3 gives their relative activity in decomposing calcium oxalate. Line 4 gives Ostwald's ('91) determinations of their relative affinities for hydroxids. Line 5 gives their relative activity in inverting cane sugar.

The correspondence of the results presented on lines 2–5 with the ionization data on line 1 is certainly quite striking. Arrhenius ('83) was the first to point out this close numerical agreement. Since the publication of his work in 1883 the idea has taken firm hold on many chemists that the ionized portion of an acid and that only is chemically active. Whetham ('95) expresses concisely recent views when he says (p. 165): "We may take it, then, that only that portion of a body is chemically active which is electrolytically active — that ionization is necessary for chemical activity just as it is necessary for electrolytic conductivity."

What applies to chemical activity must also apply to physiological activity, for in its ultimate analysis the latter is doubtless due to the former. Kahlenberg and True ('96) remark (p. 35): "It has always been taken as axiomatic that the physiological action of any substance is due to its chemical character."

The first work which deviated irreconcilably from the theory that all acids have "specific coefficients of affinity . . . . based on the fact that the relative affinities of different acids are the same, whatever the nature of the action by which they are compared" (Whetham, '95, p. 162) was that of Levy ('95). It will be seen (line 6) that acetic, mono- and dichloracetic give coefficients of activity which are in round numbers 200 per cent. in excess of that called for by the theory. The physiological activity of the acids towards phanerogams (Kahlenberg and True '96) (line 7) is equally out of harmony with the theory, when we find the almost un-ionized HCN much more active than the "strong" mineral acids. The climax, however, is reached in the data recorded on line 8, where we are dealing with concentrations which contain in many cases a very large proportion of un-ionized molecules.

The chemical reactions involved in physiological investigations are doubtless vastly more complex than in the case of the earlier studies recorded on lines 2–5. In the data recorded on lines 6–8, derived from the action of the acids on the more complex carbon compounds, and the highly complex aldehydes, albuminoids, etc., found in the protoplasm of living cells, we surely find a great exception to the alleged law that the relative affinities of different acids are the same *whatever the nature of the action by which they are compared.* These affinities, indeed, appear in some cases to be almost the converse of that required by the theory above noted.

HYDROXIDS.

As it is quite impossible to handle solutions of hydroxids in an ordinary atmosphere without a greater or less loss by neutralization by the CO_2 of the air, the following toxic values of potassium hydroxid and ammonia must be somewhat less than their absolute toxic value. This source of error was reduced as much as possible by rapid handling in making the cultures.

Potassium hydroxid, KOH; 77 (?), *166*, 282. In no other case was it found so difficult to determine where to place the coefficient of injury. $\frac{n}{512}$ retarded germination in all cases, and with some forms $\frac{n}{1024}$ and even $\frac{n}{2048}$ (Œdocephalum) showed an injurious influence.

At 24 and 36 hours, however, the cultures presented a very different appearence. Cultures in $\frac{n}{2048}$ concentration showed a heavier mycelium than the checks, and with stronger concentrations this stimulation of mycelial development was more marked until the climax was reached in $\frac{n}{64}$ or $\frac{n}{32}$ concentration. As $\frac{n}{12}$ proved the average inhibiting point, it will be seen that, as with the acids, the maximum stimulation of mycelial development occurred about two removes from the limit of germination, or, in other words, in solutions containing one fourth the concentration of the agent inhibiting the germination of the spores. The retardation of fruiting in the stimulated cultures was very marked, and suggested the query as to whether they were both due to the same cause, or whether one was a result of the other. It is well known that with the higher plants suppression of fruiting tends to force the energies of the plant into vegetative lines, and it is not apparent to the writer why this should not be true of the fungi also. On the other hand, Botrytis, which did not fruit, showed nearly or quite as great a stimulation of mycelial development as any other form.

The toxic properties of KOH are probably largely due to the $\overline{O}H$ ion. It is about 94 per cent. ionized at $\frac{n}{12}$ (Ostwald '86), its inhibiting point. Just what proportion of the toxic properties is to be attributed to the remaining 6 per cent. un-ionized KOH we have as yet no means of knowing. Inasmuch, however, as KOH is more highly ionized than HCl at the inhibiting point and is distinctly more toxic, we may safely conclude that the $\overline{O}H$ ion is somewhat more toxic for fungi than ionic $\overset{+}{H}$.

Ammonium hydroxid, NH_4OH; 29 (?), *51*, 83. This hydroxid, in contrast with KOH, is but slightly ionized, $\frac{n}{40}$, the inhibiting concentration, being about 8 per cent. ionized (Kohlrausch '85.) Its high toxic value is then doubtless due very largely to the un-ionized molecule.

Like KOH, although to a less degree, it caused a marked stimulation of mycelial development in many cultures. It is worthy of note that Sterigmatocystis, which is particularly resistant to both ionic $\overset{+}{H}$ and $\overline{O}H$, proves quite susceptible to both acids and hydroxids in the molecular form.

These results with hydroxids are not in harmony with those obtained by Krönig and Paul ('97) with anthrax spores. They found that the bases KOH, NaOH, LiOH and NH_4OH "*disinfect in direct proportion to their degree of ionization*," NH_4OH being practically non-toxic. Anthrax spores have evidently a great specific resistance to this agent, and perhaps even a general comparison would be unfair. Be this as it may, ammonia is without doubt one of the more violent poisons for fungi, far surpassing the mineral acids, copper, cobalt, etc., in toxic properties, and comparing favorably with KCN.

Data as to the effect of hydroxids on the higher plants are rather meager. Kahlenberg and True ('96) found Lupinus to survive in $\frac{n}{200}$ KOH. As ionization is practically complete here, Lupinus is evidently much more resistant to $\overline{O}H$ than to ionic $\overset{+}{H}$. Bokorny ('88) found that ammonia in $\frac{n}{689}$ concentration, in common with other basic substances, caused the production of granules in the protoplasm of Spirogyra cells, but failed to modify otherwise the normal activities of the cell. Detmoor ('94) found that a 10 per cent. solution of ammonia at first energetically excited the protoplasm of Tradescantia hairs, later producing anæsthesia. Washing with water, however, restored the original characters of the protoplasm. From these data it would seem that hydroxids are more fatal to the molds than to the higher plants.

Formaldehyde, HCHO; 0.553, *1.43*, 2. Formaldehyde, as was anticipated, proved to be one of the most deadly agents tested, being surpassed in this respect by mercury, silver, and the two chromates only. Chemically considered, formaldehyde is a very unstable compound intermediate between methyl alcohol and formic acid, being in fact the intermediate step in the oxidation process by means of which the latter is derived from the former. It is both a reducing and oxidizing agent, and this together with its great instability may account for its extremely toxic properties toward fungi.

To many kinds of protoplasm, including that of the higher animals and perhaps the higher plants, formaldehyde is nontoxic. Instances are on record of persons having drunk a 1 per cent. solution without inconvenience (Arthur '97, p. 21). To the lower animals, however, it is more toxic, $\frac{n}{60}$ being fatal to worms, mollusks and isopods in two hours (Loew '88). Acton ('89), in experiments on the assimilation of organic compounds by green plants, found that while they could use glucose, saccharin, glycerin, etc., they failed to use aldehydes or their derivatives. Cohn ('94) found a 1 per cent. solution very fatal to Spirogyra.

For the fungi, however, there is no doubt that in formaldehyde we have one of our safest, most energetic, and most serviceable poisons. $\frac{n}{512}$ proved fatal to Aspergillus and Penicillium;

$\frac{n}{2048}$, to Sterigmatocystis and Botrytis. Œdocephalum was inhibited by $\frac{n}{16384}$, and killed by $\frac{n}{8192}$. This will perhaps be better appreciated if stated in another way. One part by weight in 273,066 parts beet infusion proved fatal to Œdocephalum, and 1 part in 4,369,066 permitted the germination of but 10 per cent. of the spores in eleven hours (as compared with 95 per cent. in four hours in the checks) and greatly injured the mycelial development. In regard to the other forms, 1 part to 273,066, although greatly retarding germination, caused a distinct stimulation of mycelial growth on the second day. These stimulated cultures resembled those growing in media containing alcohol.

Very interesting in this connection are the theories regarding the synthesis of starch in green plants, and of the proteids in the fungi (Kozlowski '99), in both of which formaldehyde has long been regarded as forming a very important step. These theories, particularly that in regard to the synthesis of proteids in the fungi, challenge further careful investigation. It seems, *prima facie*, inconceivable that a compound markedly injurious to a plant when present in the almost infinite dilution of one part of weight in 4,369,000 parts nutrient medium, as is the case of Œdocephalum, should be formed by the protoplasm of that plant and be used again in the synthesis of its proteids, as must be the case if our theories be correct.

In regard to the nature of its toxic action we have few data. It is believed to act upon the propeptones and the albumins, affording compounds which are not readily soluble (Davenport '97).

Ethyl alcohol, C_2H_5OH ; 717, *3686*, 8602. Alcohol when contrasted with formaldehyde, is apparently non-toxic. One molecule of formaldehyde has powers of inhibiting germination of fungus spores equal to those of 2600 molecules of alcohol. The contrast in their killing powers is even greater, being as 1 : 4300! Yet, alcohol is a distinct poison to the protoplasm of plants (Tsukamoto '95), being used, in fact, quite extensively as a fixing agent for tissues, and even more widely as a preservative. It inhibits germination in the mold spores used at an average concentration of $\frac{n}{1.3}$, and is fatal to all except Penicillium in $\frac{4n}{1}$. Five per cent. of the spores of Penicillium survived immersion in this concentration (17.6 per cent.) for 72 hours at 28° C. All, however, were killed by $\frac{8n}{1}$ which was the greatest concentration required to kill in the case of any agent tested.

The presence of alcohol in $\frac{n}{8}$ and $\frac{n}{16}$ concentration distinctly retarded germination of Sterigmatocystis and Botrytis. No retardation was noticed with Aspergillus or Œdocephalum. The latter, indeed, showed some evidence of acceleration of germination in $\frac{n}{16}$ and $\frac{n}{32}$ concentrations, but it was not sufficiently marked to be certainly stated. The stimulation of mycelial development and retardation of fruiting in $\frac{n}{4}$ was very marked with Aspergillus and Sterigmatocystis. Œdocephalum and Penicillium showed some stimulation of mycelial development and a slight retardation of fruiting in this concentration. Botrytis produced its heaviest mycelium in $\frac{n}{16}$ and $\frac{n}{32}$.

The change that a plant may undergo as it grows older, in the character of its election of foods is of great interest to physiologists (Davenport '99, p. 333). Duclaux ('89) found that while alcohol restrains or arrests germination in mold spores, it is made use of almost as abundantly as sugar by the adult plant. My results would seem to support this view. It may be possible, however, that alcohol acts as a stimulant rather than as a food, as is the case with zinc sulfate, and other non-nourishing compounds. Richards ('97) found that the addition of .0035 per cent. $ZnSO_4$ to a culture medium in which *Aspergillus niger* was growing doubled the dry weight of the mycelium. .008 per cent. $ZnSO_4$ similarly added to a flask culture of Botrytis caused a production of quadruple the normal weight of mycelium. We cannot suppose that the small amount of zinc present in itself caused the greater growth by supplying nourishment, Zn not being a necessary or desirable element in a nutrient medium for fungi. Richards interpreted the function of the zinc to be that of a stimulant rather than a food. May it not be that alcohol performs a similar stimulating function, rather than that it produces an acceleration of growth by nourishment?

Whatever may be the correct explanation of the influence of alcohol on the development of the mold fungi, it seems to be demonstrated that the protoplasm of the molds is more sensitive in the conidial stage to the influence of this and most other deleterious agents than at any other stage in their development.

Potassium cyanid, KCN; *2.2, 25.6, 77.* Potassium cyanid in aqueous solution is very unstable. A solution of 24 per cent. KCN in pure water was prepared by the chemist, and on being used within three hours of titration gave the following critical points with Aspergillus and Penicillium:

Aspergillus in $\frac{n}{128}$ grew, in $\frac{n}{64}$ failed.

Penicillium in $\frac{n}{128}$ grew, in $\frac{n}{64}$ failed.

Ten days later this stock solution was again tested, having been kept in a dark cupboard at ordinary laboratory temperature in the meantime, with the following result:

Aspergillus in $\frac{n}{32}$ grew, in $\frac{n}{16}$ failed.

Penicillium in $\frac{n}{64}$ grew, in $\frac{n}{32}$ failed.

A solution in beet infusion containing $\frac{n}{8}$ $\mathrm{C\bar{N}}$ was then made up from the stock solution and placed in the dark at a constant temperature of 28° C. for 10 days longer and again tested. The critical points were then as follows:

Aspergillus in $\frac{n}{16}$ grew, in $\frac{n}{8}$ failed.

Penicillium in $\frac{n}{16}$ grew, in $\frac{n}{8}$ failed.

From these data we learn that a 24 per cent. aqueous solution of KCN deteriorates so that at the end of ten days it has but little more than one fourth its former toxic value. Made up in beet infusion at $\frac{n}{8}$ concentration and kept ten days longer it retains but one eighth of its original toxic value. All cultures with KCN, reported in this paper, except those noted above, were made up within four hours of titration of the stock solution.

It will be seen by reference to the charts that KCN in solution has almost exactly nine times the toxic effect of ionic $\overset{+}{H}$. KCN in the concentrations used is quite highly ionized (Kohlrausch '79), but in trying to approximate the toxic value of the $C\overline{N}$ ion, the fact that a certain amount of hydrolysis takes place in aqueous solutions of this salt, with a corresponding formation of the deadly HCN, must not be overlooked. According to the data worked out by Shields ('93) and what we already know of the properties of HCN, approximately 15 per cent. of the total toxic value of these solutions must be attributed to the HCN present. This would give something under $8\overset{+}{H}$ as the value of the $C\overline{N}$ ion.

KCN retarded germination and early development in all forms. In this, as with many other agents, those cultures not greatly injured soon overcame the effect of the poison and grew and fruited normally. No marked retardation of fruiting nor unusual development of mycelium was noted. Sterigmatocystis, in so many cases highly resistant, proved equally sensitive with Œdocephalum, both being inhibited by $\frac{n}{128}$ and killed by $\frac{n}{64}$.

Kahlenberg and True ('96) found that towards Lupinus it has the value of $1\overset{+}{H}$ only. They also show that in the cases of potassium ferro- and ferri-cyanid the iron and $C\overline{N}$ radical form complex ions, the toxic value of which is far less than that of the $C\overline{N}$ ions.

Mercuric chlorid.—$HgCl_2$; 0.0258, *0.281*, 0.331. This proved the most fatal compound tested, leading silver nitrate by a narrow margin.

It is of interest to note that although only very slightly volatile at ordinary temperatures, and doubtless less so in aqueous solution, mercuric chlorid is sufficiently volatile even in dilute aqueous solution at 28°C. to be distinctly toxic. This was demonstrated by placing a few drops of a very dilute solution in the bottoms of cells containing hanging drops of pure beet infusion inoculated with mold spores. The germination of the spores was inhibited. Mycologists have frequently reported failure to germinate spores in cells which had been sterilized by rinsing in a dilute solution of $HgCl_2$. These failures were doubtless due to the volatile properties of this agent together with its extremely deadly character.

Botrytis proved particularly sensitive to this agent, $\frac{n}{65536}$ proving fatal. Penicillium failed to show its usual high relative resistance, being killed by $\frac{n}{4096}$, the same concentration as proved fatal to Aspergillus and Sterigmatocystis. Toward bacteria it is also extremely fatal, $\frac{n}{70000}$ in nutritive bouillon preventing the development of the splenic fever bacterium (Davenport '97, p. 14). The data regarding its influence on the higher plants are meager. Kahlenberg and True ('96) found $\frac{n}{6400}$ fatal to Lupinus, while it survived in $\frac{n}{12800}$. This is unexpectedly low, being in fact but double the toxic value of HCl. For the molds its average value will be seen to be over 800 times that of HCl.

Silver nitrate, $AgNO_3$; 0.0125, *0.375*, 0.375. Almost, if not altogether, as violent a poison as mercury, silver stands with it at the head of the list of toxic agents tested. Among the poisons for molds tested, it is comparable with mercury alone among the metals, and with the chromate and dichromate anions and formaldehyde only among the other agents. As is the case with bacteria (Davenport '97, p. 14) toward the molds silver is frequently a more violent poison than mercury. Of the five molds used Œdocephalum and Penicillium proved more susceptible to silver, while Aspergillus and Botrytis were more susceptible to mercury. The fifth form, Sterigmatocystis, had an equal resistance to both. The extraordinarily small quantity of mercury required to kill Botrytis, however, left the honors with mercury as the more deadly agent.

A very striking contrast in specific resistances is afforded by Botrytis and Penicillium in solutions of these agents. One sixteenth the amount of $HgCl_2$ necessary to kill Penicillium was fatal to Botrytis. With $AgNO_3$ one fourth the concentration required to kill Botrytis was fatal to Penicillium. Or, putting it another way, $AgNO_3$ has eight times the toxic value of $HgCl_2$ toward Penicillium, while the exact converse is true with Botrytis, $HgCl_2$ being eight times as effective as $AgNO_3$ to this form. The low resistance of Penicillium to silver is quite striking, $\frac{n}{32768}$ proving fatal. In one other case only (H_2O_2) did Penicillium show a lower resistance than Botrytis.

Toward splenic fever bacteria gold is the only other metal comparable in toxic properties with mercury and silver (Davenport '97, p. 14). Toward phanerogams silver is much more toxic than mercury, $\frac{n}{102400}$ being fatal to Zea, and $\frac{n}{204800}$ to Lupinus (Heald '96, p. 152). At the concentrations used the $AgNO_3$ would be practically entirely ionized (Kohlrausch, '85).

Cadmium nitrate, $Cd(NO_3)_2$; 0.075, *6.1*, 24. Ranking closely with silver as a poison for the higher plants, cadmium proves to be very highly toxic to the mold fungi. Perhaps the most marked feature in the cultures with cadmium was the very wide range between the killing point and the point where development was only noticeably injured. It will be noticed that the ratio of its coefficient of injury to that of $HgCl_2$ is less than 3 : 1, while the ratio between their death points is 75 : 1. A second striking feature of the toxicity of cadmium is the great variation in the specific resistances of the different forms. $\frac{n}{4096}$ proved fatal to Botrytis, but $\frac{n}{32}$ was required to kill Sterigmatocystis. Penicillium spores failed again to show their usually high resistance, $\frac{n}{256}$ proving fatal, a concentration in which the usually sensitive Œdocephalum actually germinated 95 per cent. and matured a few fruits.

Molisch ('94) was one of the first to record the toxic properties of cadmium to plants. He found $\frac{n}{33}$ fatal to Aspergillus when experimenting with various metals in an endeavor to find a substitute for calcium in nutrient media. $\frac{n}{64}$ proved fatal to the form of Aspergillus used by the writer.

At its average inhibiting concentration cadmium nitrate would be about 90 per cent. ionized (Grotrian, '83).

OXIDIZING AGENTS.

Potassium dichromate, $K_2Cr_2O_7$; 0.094, *0.3*, 1.25.

Potassium chromate, K_2CrO_4; 0.156, *0.4*, 2.25.

These salts at the dilutions at which they are effective are doubtless practically entirely ionized (Ostwald, '88).

As poisons for the molds they rank, as already mentioned, with formaldehyde, silver, and mercury. The anion of the dichromate, $^{-}Cr_2O_7^{-}$ has a toxic value of about 770 $\overset{+}{H}$; that of the chromate 575 $\overset{+}{H}$. This may indicate some relation between their oxidizing powers and their toxicity.

The effects of these salts in concentrations permitting development of the fungi were very similar, and resembled that of H_2O_2. Retardation of germination in the cultures approaching the inhibiting point was noticed in all forms with both agents, but it was not nearly so well marked as it is in most cases. Another feature was the fact that every culture that germinated any spores developed some conidia within forty-eight hours.

Toward the higher plants these anions seem to be relatively much less toxic. Lupinus survives in a $\frac{n}{6400}$ solution of H_2CrO_4, the same concentration as permitted growth with HCl (Kahlenberg and True, '96). Toward the algæ (Loew, '93), however, both these anions are strongly toxic, $\frac{n}{295}$ $K_2Cr_2O_7$ being fatal to Spirogyra in a few hours.

Hydrogen peroxid, H_2O_2; 38, *105*, 127. This agent had an effect on the molds very similar to that described for the chromates. With it, however, the characteristics given for the chromates were somewhat intensified. Germination was but slightly retarded in most forms in $\frac{n}{312}$ concentration, and Œdocephalum actually showed a higher percentage germinated in these cultures at four hours than in the checks. The difference, however, was not sufficient to establish the conclusion that the H_2O_2 accelerated germination. That this concentration accelerated early mycelial growth with this form was undoubtedly established. At four hours the average length of the germ tubes in $\frac{n}{312}$ concentration was 120μ as compared with 40μ in the checks. At seven hours they were 310μ and 115μ respectively. $\frac{n}{156}$, the limiting culture for this form, made the best mycelial development and matured the heaviest crop of conidia in the set. The characteristic already mentioned for the chromates regarding fruiting in the cultures was even more marked with this agent. Every culture that produced even the scantiest mycelium presently developed at least two or three all but normal conidiophores.

In regard to the action of this agent on other organisms, the data are meager and conflicting. Miquel ('83) places it third in disinfecting properties of all agents used by him. In his results it is rated above both $HgCl_2$ and $AgNO_3$, being given as antiseptic in dilution of 1 part to 20,000. This is certainly too high an estimate. Sternberg ('92) found it to have a comparatively low toxic value for bacteria. One in 1000 kills ordinary water bacteria, cholera, and typhoid (Altehofer '90). This would be about $\frac{n}{33}$, or a 3 to 4 per cent. solution of the ordinary 10-volume commercial article. It will be seen that this agrees fairly closely with its toxic properties for the molds, $\frac{n}{39}$

being fatal to Sterigmatocystis and $\frac{n}{78}$ to Œdocephalum. The others are more resistant. It is, however, more toxic to algæ (Bokorny, '86) than to molds, and Ciliata are even more susceptible. Paneth ('89) found a .005 per cent. solution to be the limiting line for the latter.

Commercial preparations of H_2O_2 vary very greatly in the amount of H_2O_2 in solution. A true ten-volume solution should yield, when fully decomposed, ten volumes of O, and should contain by weight 3.04 per cent. H_2O_2. The preparation used in this study although "fully guaranteed," etc., contained but 2.59 per cent. H_2O_2 on being tested.

SULFATES OF THE STRONGLY-TOXIC METALS.

These salts are arranged in the order of their toxic properties towards molds in the following list:

Nickelous sulfate, $NiSO_4$; 4.8, *33.6*, 1155.
Cobaltous sulfate, $CoSO_4$; 6, *57.6*, 389.
Ferrous sulfate, $FeSO_4$; 14.4, *115*, 2150.
Copper sulfate, $CuSO_4$; 8.4, *131.2*, 582.
(Copper nitrate, $Cu(NO_3)_2$; 8.4, *134*, 634.)
Zinc sulfate, $ZnSO_4$; 26.4, *602*, 3072.

The data regarding the ionizations of these salts are rather meager. They are, however, not greatly different in ionization at similar concentrations. This is about 40 per cent. to 44 per cent. at $\frac{n}{20}$ concentration (Whetham, '95, pp. 218–276).

Nickelous sulfate.—The different molds exhibited more variations in their specific resistance to this agent in regard to the death point than was observed with any other. $\frac{n}{128}$ proved fatal to Botrytis, while Aspergillus failed to lose its vitality in a normal solution (containing over 13 per cent. anhydrous $NiSO_4$) for 48 hours. Much less variation was shown in its inhibiting powers. Aspergillus and Penicillium germinated in $\frac{n}{64}$; $\frac{n}{256}$ inhibited Botrytis. The fact that 32 times the strength which inhibited the spores of Aspergillus failed to kill them was nowhere else paralleled with this form and but once surpassed by Penicillium.

Cobaltous sulfate stands in second place in this group as an inhibiting agent, but, as will be seen, it is relatively much more powerful as a disinfectant. Penicillium, however, showed its usual high powers of resistance in this respect. Inhibited by $\frac{n}{64}$, $\frac{n}{2}$ was required to kill.

Ferrous sulfate.—Iron, a necessary element for the nutrition of the molds (Molisch, '94) in common with all other plants, in excess proves to be a very strongly toxic agent, surpassing in this respect that king of modern fungicides, copper. With the exception of nickel as noted above, iron showed a greater difference between the average concentration required to inhibit the spores and the concentration required to kill than any other agent. Botrytis, as usual, showed less variation in this respect than the other forms, but even with it one eighth the fatal concentration inhibited germination. Œdocephalum showed the greatest resistance to this agent both as regards inhibition of germination and killing of the spores. This was the only agent with which it had a higher specific resistance than any other form.

Copper sulfate and nitrate.—These salts proved to be quite similar in toxic properties, as may be noticed by a glance at the diagrams, p. 312. The nitrate, however, is much more highly ionized at the critical concentrations; hence we judge that the un-ionized molecule $CuSO_4$ has a toxic value not greatly different from ionic $^+Cu^+$.

Penicillium, although inhibited by $\frac{n}{64}$ $Cu(NO_3)_2$ and $\frac{n}{128}$ $CuSO_4$, required a $\frac{n}{1}$ concentration in both cases to kill the spores. This certainly shows great resistance to these agents as compared with the other molds. It, however, appears insignificant when contrasted with many of the results gotten by other workers. $\frac{n}{128}$

$CuSO_4$, which effectually inhibited germination in the form used by the writer, contains about 0.1 per cent. $CuSO_4$. De Seynes ('95) reports growing cultures of *Penicillium glaucum*, gotten from different sources, in solutions containing 2 to 9.5 per cent. $CuSO_4$. Cultures grown on the stronger concentrations bore red spores. Pfeffer ('81) reports finding Penicillium growing on a concentrated solution of $CuSO_4$. Manasein (*fide* Loew '93) finds from his experiments that this salt must be present in a .25 per cent. concentration before it has any appreciable effect on this fungus. Others might be quoted, but sufficient has been said to indicate the possibilities yet to be investigated of the acclimatization of fungi (and other plants) to chemical agents (Davenport and Neal, '96).

Zinc sulfate.—Inasmuch as zinc chloride is used very extensively for impregnating railroad ties to prevent attacks of wood-destroying fungi (Roth, '95), it was a surprise to find it having so low a toxic value, particularly when it is recalled that one of the molds tested, Penicillium, is one of the enemies of the wooden ties (Ward, '98). Koch ('81) finds the chlorid and the sulfate to have practically the same disinfecting power. Towards Aspergillus we may say that zinc is non-toxic, the spores surviving an immersion of 48 hours in a $\frac{2n}{1}$ (27 per cent. anhydrous $ZnSO_4$) concentration. In a $\frac{n}{2}$ concentration (7 per cent.) 25 per cent. of the spores germinated and grew slowly. The mycelium produced was very irregular and closely septate.

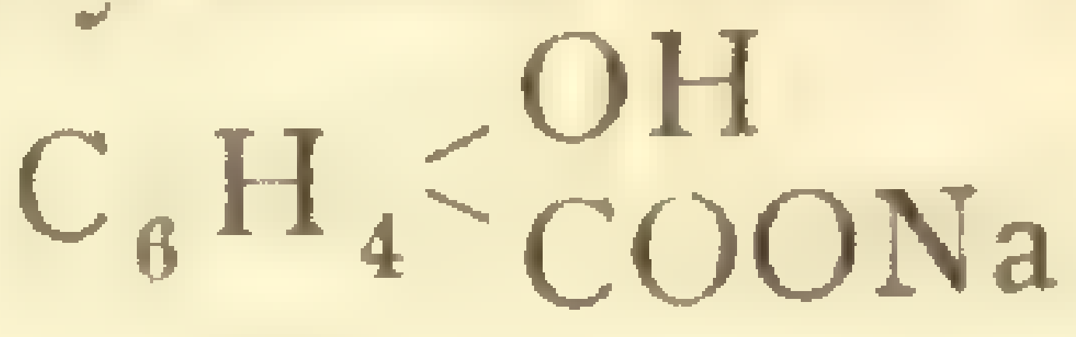
Strychnin sulfate, $C_{21}H_{22}N_2O_2H_2SO_4$. This alkaloid, the only one experimented upon, was dissolved in the slightly acid beet infusion until a saturated solution was obtained. This was found to have a content of 4.31 per cent. strychnin sulfate. This in terms of a normal solution would be about $\frac{n}{7}$, a normal solution of this substance requiring over 30 per cent. on account of its very large and heavy molecule.

Sterigmatocystis grew and fruited normally in this saturated solution, although germination and early growth were retarded.

Aspergillus and Œdocephalum also grew and fruited, but the cultures were much behind the checks. Penicillium was inhibited by $\frac{n}{28}$, but was not killed by the saturated solution. Botrytis was also inhibited by $\frac{n}{28}$, and was killed by $\frac{n}{14}$. That the solution used was completely saturated was shown by the appearance of numerous microscopic crystals in some of the hanging-drops which were exposed for a few moments to the air, the evaporation from the culture medium causing some of the strychnin to crystallize out. The molds, however, continued to thrive in these cultures, their hyphæ growing among the crystals.

Of its effect on plants in general we have few data. Davenport ('97) mentions that it kills the protoplasm of Drosera tentacles, and hinders the development of peas, corn, and lupines. The injurious concentrations, however, are not mentioned. Much interesting work has been done on Protozoa (Schürmayer '90) by various workers. The results of these studies as well as those presented here for the molds are in harmony with the theory of Loew ('93) that the action of alkaloids is chiefly confined to the plasma of the ganglion cells. Fungi and bacteria having no differentiation of nerve protoplasm are practically unharmed by this agent.

Potassium iodid, bromid, and chlorid.—These salts proved to have a very low toxic value. A complete series of cultures with the five molds was made up with the iodid only. Its coefficients were determined to be 384, *2457*, and 4915. $\frac{n}{1}$ inhibited all except Aspergillus, $\frac{2n}{1}$ was fatal to all except Penicillium. As the potassium salts of the haloid acids are all quite highly ionized, an attempt was made to determine the relative toxic properties of the *ionic* halogen elements. Aspergillus and Œdocephalum were used. To these molds ionic $\bar{\mathrm{I}}$ proved doubly toxic as compared with $\overline{\mathrm{Cl}}$. $\overline{\mathrm{Br}}$ occupied an intermediate position, being very slightly more toxic than ionic $\overline{\mathrm{Cl}}$.

Sodium salicylate  $C_6H_4{<}\begin{matrix}\mathrm{OH}\\ \mathrm{COONa}\end{matrix}$; 24, *182*, 182. It was thought desirable to test this salt on account of its wide use as a preservative in laboratories and elsewhere. It proved somewhat more toxic than HCl, but not so fatal as HNO_3. Œdocephalum was quite susceptible to its influence, $\frac{n}{128}$ being fatal. It is not, however, of much value as a disinfectant, over 1 per cent. being necessary to prevent the development of molds.

In the following table the various agents are arranged in the order of their toxic properties as shown by their powers of inhibiting germination of the spores of the five species of molds used. The fifth column gives in round numbers the number of molecules of each substance required to produce an inhibiting effect equal to that *of one molecule of mercuric chlorid.* The coefficients have the usual value of x in the expression, $\frac{x}{2048}$ of $\frac{n}{1}$.

TABLE III.

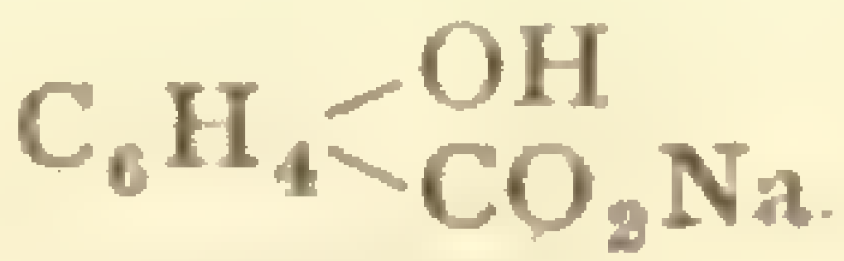
Agent	Formula	Coefficient of injury	Coefficient of inhibition	Coefficient of death-point	Ratio
Mercuric chlorid	$HgCl_2$	.026	.281	.331	1.
Potassium dichromate	$K_2Cr_2O_7$	.094	.3	1.25	1.1
Silver nitrate	$AgNO_3$	.013	.375	.275	1.3
Potassium chromate	K_2CrO_4	.156	.4	2.25	1.4
Formaldehyde	$HCHO$	.553	1.43	2.	5.
Hydrocyanic acid	HCN	.365	3.	20.	11.
Cadmium nitrate	$Cd(NO_3)_2$	.075	6.1	24.	22.
Potassium cyanid	KCN	2.2	25.6	77.	91.
Nickelous sulfate	$NiSO_4$	4.8	33.6	1155.	120.
Ammonium hydroxid	NH_4OH	8.	51.	83.	182.
Cobaltous sulfate	$CoSO_4$	6.	57.6	389.	206.
Monochloracetic acid	CH_2ClCO_2H	8.8	58.	64.	207.
Dichloracetic acid	$CHCl_2CO_2H$	10.4	64.	64.	229.
Acetic acid	CH_3CO_2H	25.6	83.	314.	296.
Trichloracetic acid	CCl_3CO_2H	37.	90.	115.	321.
Hydrogen peroxid	H_2O_2	38.	105.	127.	375.
Ferrous sulfate	$FeSO_4$	14.4	115.	2150.	411.
Copper sulfate	$CuSO_4$	8.4	131.	582.	468.
Copper nitrate	$Cu(NO_3)_2$	8.4	134.	634.	479.
Nitric acid	HNO_3	48.	141.	384.	503.
Potassium hydroxid	KOH	19. 77.	166.	282.	593.
Sodium salicylate	 $C_6H_4{<}\begin{matrix}OH\\CO_2Na\end{matrix}$	24.	182.	182.	650.
Sulfuric acid	$\frac{1}{2}H_2SO_4$	61.	205.	589.	732.
Hydrochloric acid	HCl	70.	230.	614.	821.
Zinc sulfate	$ZnSO_4$	26.4	602.	3072.	2150.
Strychnin sulfate	$C_{12}H_{22}N_2O_2H_2SO_4$	179.			
Potassium iodid	KI	384.	2457.	4915.	8775.
Ethyl alcohol	C_2H_5OH	717.	3686.	8602.	13164.

DIAGRAM IX.

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2
1
$HgCl_2$
$K_2Cr_2O_7$
$AgNO_3$
K_2CrO_4
HCHO
HCN
$Cd(NO_3)_2$
KCN
$NiSO_4$
NH_4OH_4
$CoSO_4$
CH_2ClCO_2H
$CHCl_2CO_2H$
CCl_3CO_2H

DIAGRAM X.

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600
575
550
525
500
475
450
425
400
375
350
325
300
275
250
225
200
175
150
125
100
75
50
25
HCN
KCN
NH_4OH
$CoSO_4$
CH_2ClCO_2H
$CHCl_2CO_2H$
CH_3CO_2H
CCl_3CO_2H
H_2O_2
$CuSO_4$
HNO_3
KOH
$C_6H_4 < \begin{matrix} OH \\ COONa \end{matrix}$
$\frac{1}{2}H_2SO_4$
HCl
$ZnSO_4$

Diagrams IX and X are simply a graphic representation of the more important features of this table. The values of the abscissæ in each case $= x$ in the expression $\frac{x}{2048}$ of a normal solution.

SUMMARY.

1. Fungi are in general much more resistant to most deleterious agents than the higher plants. In the case of the mineral acids a concentration of from two to four hundred times the strength fatal to the higher plants is required to inhibit the germination of mold spores under favorable conditions.

2. Different species of fungi present great differences of resistance to many agents. Of the agents tested in this study, $NiSO_4$ permitted the greatest specific variation and dichloracetic acid the least.

3. Particular forms of the same species present very different powers of resistance, depending probably on previous environment.

4. Individual spores taken from the same pure culture often present considerable variation in resistance.

5. The five forms used were found to be increasingly resistant to the toxic action of acids in the following order: Œdocephalum, Botrytis, Penicillium, Aspergillus, and Sterigmatocystis.

6. Toward all the agents tested they proved increasingly resistant in the following order: Botrytis, Œdocephalum, Penicillium, Sterigmatocystis, Aspergillus.

7. Œdocephalum and Botrytis, although on the average the most susceptible to the various agents, have great specific resistances to certain agents. See $FeSO_4$, KI, and alcohol.

8. Tests made with media not well suited for the normal development of the fungi tested will give a correct value for the *killing concentration*, but the data regarding the point of inhibition of germination are not of value.

9. Tests of the toxic value of solutions are unreliable when made in hanging-drop cultures where water was used in the

bottom of the cell instead of a solution similar in composition to that forming the hanging-drop. The variation in the toxic value indicated from the actual value will depend on the *vapor pressures* of the solutions used. Volatile — especially highly volatile — and hygroscopic solutions will show the greatest error.

10. Many deleterious agents which at certain concentrations retard germination and early growth, afterwards cause a great acceleration of mycelial development in these retarded cultures. This abnormal development of mycelium is usually accompanied by retardation of fruiting.

11. In the conidial stage the protoplasm of molds is in general more sensitive to the action of deleterious agents than at any other stage in their life history.

12. The effect of the different deleterious agents on the appearance of the mycelium is very varied and often quite characteristic.

13. One is not justified in drawing any conclusions as to the killing powers of an agent from its effect in inhibiting the germination of the spores.

14. The hydroxyl group $\overset{-}{O}H$ is rather more toxic to molds than ionic $\overset{+}{H}$.

15. The toxic value of the halogens, Cl, Br, and I, in the *ionic* state, increases somewhat in the order of increasing atomic weight.

16. The cyanogen radical is a very powerful poison to fungi, KCN having nine times the toxic value of HCl.

17. Mercuric chlorid and silver nitrate are about equally toxic to molds; and are followed in toxic properties by potassium dichromate and chromate, and formaldehyde.

18. Strychnin and hydrocyanic acid, both extremely fatal poisons to the higher animals, and both supposed to act on the protoplasm of the nerve cells, react very differently toward fungi. To the molds strychnin is practically non-toxic, whereas hydrocyanic acid is a very violent poison.

19. Nickel, cobalt, iron, copper, and zinc inhibit mold spores in the order named. Zinc is much less toxic than the others.

20. That an element is necessary for the nutrition of a plant does not indicate whether it would or would not be a poison in greater concentration. See iron, oxygen, etc.

21. That an element is not necessary for the normal development of a plant does not imply that it would be injurious even in relatively great concentration. See chlorin, calcium, etc.

22. The ionization of the molecule of electrolytes in aqueous solution has a very important bearing on the study of the physiology of poisons. It is of especial value in determining the element or group of elements in a compound to which its toxic properties are to be attributed.

23. In this study no new evidence has been adduced supporting the theory that the chemical activities of a substance are due wholly or chiefly to the ionized portion.

24. Evidence has been adduced to the effect that in the case of several acids ionization *lessens* the chemical activities toward the substances involved in the life processes of the plant.

25. In the case of the eight acids investigated six were found to be much more toxic in the molecular form than after ionization. The toxic properties of the un-ionized molecules vary from approximately 2.8 times that of ionic $\overset{+}{H}$ in the case of acetic acid to 76.6 times that of $\overset{+}{H}$ in hydrocyanic acid.

26. The substitution of Cl for H in the acetic acid radical has a double effect. In the first place it increases the toxicity of the un-ionized molecules to a greater or less extent depending on the number of H atoms so replaced. In the second place, it increases the ionization of the acid. The amount of the ionization is also dependent on the amount of H so replaced, being greatest, as are the toxic properties of the whole molecules, when all three H atoms have been replaced by Cl.

27. These two factors to a great extent counterbalance each other. Which has the greater influence in any given solution depends altogether on the concentration, the increased toxicity of the molecules having the predominating influence at the greater concentrations, and the ionization being more effective at the greater dilutions.

28. At the concentrations inhibiting fungus spores mono- and dichloracetic acids are more influenced by the increased toxic properties of the molecule, and trichloracetic by the ionization. The former in $\frac{n}{35}$ and $\frac{n}{32}$ concentration are respectively increased in toxicity 40 per cent. and 30 per cent. over the original acetic. Trichloracetic, on the contrary, at $\frac{n}{22.8}$ suffers a reduction of 10 per cent. in toxic properties as compared with the original acetic.

29. The anions of the mineral acids, HCl, HNO_3, and H_2SO_4, have a low toxic value for fungi, having less than one thirty-second that of ionic H.

In conclusion I wish to acknowledge my indebtedness to Dr. B. M. Duggar, instructor in plant physiology, and Professor George F. Atkinson, professor of botany in Cornell University, for much valuable advice and assistance, and constant encouragement. My best thanks are also due to Professor W. D. Bancroft and Mr. A. L. Knisely of the chemical department for much help and information on the chemical aspects of the work.

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STUDIES IN CRATÆGUS. I.

C. D. BEADLE.

FEW genera so widely distributed in the United States have been so poorly interpreted by American botanists as the genus Cratægus. In Europe there exists a better understanding of a number of American forms, a knowledge gained almost wholly from cultivated specimens, but it must be evident to any student of these interesting plants that all of the descriptions published up to the present time fall short of embracing the forms growing in almost any section of the country. My attention was first drawn to the thorns about ten years ago, when I attempted to raise many thousands of young plants from seeds gathered in the southern Alleghany region. Making no attempt to propagate other than thrifty plants of the well-known and widely recognized species, I was perplexed to find a nursery of the most diverse forms under almost every label. Low and bushy plants with dark foliage stood in bold contrast with tall and fastigiate individuals with leaves of different tint and outline. No explanation seemed more reasonable than that some careless gardener had mixed the seeds so painstakingly collected from fine, healthy individuals, and with a determination that more care should be exercised in succeeding attempts, the matter was dropped. The next autumn the sowing was most carefully done, and while the fruits and seeds did not all look quite alike, it was easily demonstrated that the species recognized in our botanical field-books were not confounded. The results of this seeding were even more confusing than the first, as the seeds were gathered from wider fields. And thus the evidence accumulated each year, until, almost unconsciously, I commenced to separate the forms as they grew and the seeds as they were gathered. This solution was complete, and as the oldest seedlings are now bearing the kinds of fruits earlier recognized as strange, the publication of new species is fully justified. In this, and other papers which will appear as suitable material accumulates, I propose to deal with new forms coming to my attention, and to characterize the published species, with which are now confounded others with constant and widely different features.

Cratægus Biltmoreana, n. sp.—A branching shrub, 1–5^{m} tall, growing in dry or rocky woodlands: flowers, which appear when the leaves are almost fully grown, white, 2–2.5^{cm} in diameter when expanded, produced in simple, pubescent, 3–7-flowered corymbs with lanceolate, pectinately-glandular, caducous bracts; they are borne on strict, pubescent pedicels which vary from 7^{mm}–2.5^{cm} in length and open in the vicinity of Biltmore, N. C. (type locality), about the twentieth of May: calyx obconic, pubescent or tomentose on the outside, with lanceolate, dentate or pectinate, glandular lobes about 5^{mm} long which are reflexed after anthesis: petals broadly-obovate or nearly orbicular in outline, 8–12^{mm}, contracted near the base into short claws: stamens 10, shorter than the petals; the anthers pale yellow: styles 3–5, shorter than the stamens, surrounded at the base with pale hairs: fruit, which ripens and falls at the end of September or early in October, yellow, greenish-yellow or sometimes orange, the parts exposed to the sun being rosy-cheeked or diffused with red, containing from 3–5 nutlets; they are depressed-globose, bluntly angled, 10–15^{mm} broad, 10–12^{mm} high, the cavity broad, 3–5^{mm}, and deep, 2–3^{mm}, surrounded by the calyx lobes and portions of the filaments: nutlets thick walled, 5–7^{mm} in greatest diameter, 3–5^{mm} measured dorsoventrally, a volume of 125^{cc} containing about 1858 thoroughly cleaned, dry seeds; they are deeply grooved on the back and display a prominent ridge near the middle, the inner faces being nearly plane: leaves 2–6^{cm} wide, 3–10^{cm} long including the petiole, or occasionally larger, ovate, or round-ovate, acute at the apex; rounded, truncate or on vigorous shoots subcordate, but usually wedge-shaped at the base and prolonged into a slightly winged or margined petiole 1–3^{cm} long and bearing, as does the extreme base of the leaf, a few dark-colored stalked glands; borders acutely incised, or slightly 5–9-lobed and sharply and irregularly serrate to near the base; they are thin and membranaceous at flowering time, becoming firmer and thicker with age, harshly, though rather inconspicuously pubescent on both surfaces throughout the vegetating season, bright green on the upper surface, pale beneath, the prominent veins being disposed in 4–6 pairs: spines stout, 2–5^{cm} long on the branches, slightly curved, dark chestnut-brown on the new and gray on the old wood: bark of the main stem reddish-brown, slightly fissured and broken into many small, persistent, ashy-gray scales; that of the branchlets chestnut- or reddish-brown, or gray, sprinkled with small, pale lenticels: buds almost globular, bright reddish-brown.

Cratægus Biltmoreana is distributed from North Carolina, northern Alabama, and eastern Tennessee to Virginia and Pennsylvania. It has been usually confounded with and preserved in herbaria under the name *C. mollis* (T. & G.) Scheele,[1] from which, or any form now resting under this name, the Biltmore thorn may be known by its smaller size, simple corymbs, later time of blossoming, and by the color and texture of the fruit. The type material is preserved in the Biltmore Herbarium.

Cratægus Sargenti, n. sp.—An intricately branched tree, seldom more than 6^{m} tall, or more frequently a large shrub 2-5^{m} in height, with one or several stems: the bark of the trunk ashy-gray or light brown, slightly fissured and broken into many thin plate-like scales, or nearly smooth with scattered patches of appressed, small scales: branches spreading or ascending, armed with straight or curved, simple or branched, dark chestnut-brown or gray spines 2.5-7^{cm} long; they are intricately divided into numerous, short branchlets which are clothed with dark reddish-brown bark and marked by small round or elongated pale lenticels, forming a narrow, or occasionally round or flat-topped head: buds globular, bright reddish-brown: flowers, which appear about the first of May in the vicinity of Valley Head, Alabama, and when the leaves are almost fully grown, disposed

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[1] Linnæa **21**: 569. 1848.

in a few- (mostly 3-) flowered, more or less pubescent, simple corymbs: lateral pedicels longer than the intermediate ones, 1.5–3.5^{cm} long, more or less pubescent or pilose: calyx obconic, pubescent, the segments glandular-serrate, 6–9^{mm} long, persistent or nearly so: corolla white, the divisions nearly round or a little broader than long: stamens normally 20, 5–7^{mm} long: pistils 3–5, surrounded at the base with pale hairs: fruit, which ripens and falls after the middle of September, globose or depressed-globose, 10–13^{mm} broad, 10–12^{mm} high, yellow, orange-yellow or flushed with red, the flesh thin and firm; cavity 3–5^{mm} broad and nearly as deep, surrounded by the remnants of the stamens: nutlets 3–5, but usually 4, hard and bony, the walls thick, 7–9^{mm} long, 4–6^{mm} measured dorso-ventrally, with the back ridged and grooved and the lateral faces nearly plane: leaves thin to subcoriaceous, sparsely pubescent when young, soon glabrous, yellowish-green on the upper surface, paler below and displaying 5–7 pairs of prominent veins; they are ovate, ovate-lanceolate or round-ovate, 2.5–12^{cm} long, 1–6^{cm} wide, or occasionally larger on vigorous shoots, acute at the apex, rounded or abruptly contracted at the base into a margined or winged, slightly glandular petiole, 5^{mm}–3.5^{cm} long, the borders irregularly and doubly serrate and incisely lobed and the serratures minutely glandular-tipped: stipules linear to linear-lanceolate, glandular, or on strong shoots foliaceous, lunate, glandular-serrate, caducous.

Cratægus Sargenti is a remarkably distinct and showy species, especially in the autumn when the foliage assumes many lively tints of red and yellow. It inhabits the rocky woods and bluffs, or occasionally the rich, deep soil of the mountainous regions of northwestern Georgia, northern Alabama (extending as far south as Birmingham), and southeastern Tennessee. The species belongs to an interesting and very natural group of several distinct species of which no type has, so far as I have observed, been published. Many specimens of the related forms are preserved in herbaria under the names *C. rotundifolia*, *C. glandulosa*, *C. coccinea*, etc., titles which are, when correctly applied, associated with widely different plants.

I take pleasure in naming the species in honor of Professor C. S. Sargent, Director of the Arnold Arboretum of Harvard University, who first called my attention to specimens collected by him near Rome, Georgia, in April 1899. The type material, which I had the opportunity of selecting from thousands of examples near Valley Head, Alabama (type locality), is preserved in the Biltmore Herbarium.

Cratægus Boyntoni, n. sp. — A tree seldom more than 6^{m} in height, or more frequently a large branching shrub, $2–4^{m}$ tall, frequenting the banks of streams and even the shallow, dry soil of old fields and upland woods: flowers, which expand about the tenth of May in the vicinity of Biltmore, N. C. (type locality), and when the leaves are almost fully grown, $1.75–2.25^{cm}$ in diameter, produced in short, glandular-bracteate, 4–10-flowered corymbs: pedicels $7^{mm}–1.5^{cm}$ long, glabrous, bearing one or two glandular or pectinately-glandular bractlets: calyx obconic, smooth, the divisions acute, glandular serrate, $4–6^{mm}$ long: petals white, nearly orbicular, or a little broader than long, with a short and broad claw at the base, $9–12^{mm}$ in diameter: stamens 10, $6–9^{mm}$ long, the anthers light yellow: pistils 3–5, surrounded at the base with pale hairs: fruit dull, yellowish-green, flushed with russet-red, depressed-globose, angled, $10–14^{mm}$ high, $12–16^{mm}$ broad, ripening and falling early in October: nutlets 3–5, hard and bony, $6–8^{mm}$ long, $4–5^{mm}$ measured dorso-ventrally, the back ridged and grooved and the lateral faces nearly plane, a volume of 125^{cc} containing about 1293 thoroughly clean and dry seeds: leaves at first membranaceous, becoming subcoriaceous with age, yellowish-green on the upper, paler on the lower surface, glabrous, or with a few scattered hairs along the midrib and larger veins, which are disposed in 4–7 pairs; they are broadly ovate or oval in outline, acute at the apex, rounded or narrowed at the base and prolonged into a margined, glandular petiole $1–2.5^{cm}$ long, or on vigorous shoots deltoid-ovate and truncate or subcordate at the base; the borders are sharply and irregularly serrate, doubly serrate or incisely 5–7-lobed, the serratures minutely gland-tipped: stipules linear, glandular, caducous, or on strong shoots foliaceous, lunate, glandular-serrate: trunk, which is 2–3^{m} long and 1–2^{dm} in diameter, and occasionally armed with gray, branched spines, covered with ashy-gray bark, not infrequently tinged with brown, or in the shade of the forest dark-gray, slightly fissured and broken into many small, plate-like scales: branches stout, ascending, armed with dark, chestnut-brown or gray, straight or slightly curved spines, 3–7^{cm} long, or larger, the bark close and smooth, dark-gray or brown: branchlets smooth, the bark dark reddish-brown, sprinkled with small, round or elongated, pale lenticels, the whole forming a narrow or occasionally round or flat-topped tree, or a much-branched oval or pyramidal shrub.

Cratægus Boyntoni is distributed from Georgia, Alabama, and Tennessee to Virginia, Pennsylvania, and Delaware, and will doubtless be found to extend over a broader area when better known. It is closely related to *C. Sargenti*, above proposed, but may be separated by the numerous-flowered, glabrous corymbs, shorter pedicels, and fewer stamens, and by the different habit of growth. Many specimens are preserved in herbaria, the greater part of which are also labeled *C. coccinea*, *C. glandulosa*, or *C. rotundifolia*. My attention was first directed to this form by Mr. F. E. Boynton, of the Biltmore Herbarium, for whom the species is named, by the exhibition of plants loaded with the characteristic and distinct fruit, and of branches displaying the glandular and brightly colored characters of the unfolding leaves and shoots. To this species I refer, in part, the material representing the southern distribution of *C. rotundifolia* of the Illustrated Flora[2], and the note under this name published by me in the BOTANICAL GAZETTE[3]. The type material is preserved in the Biltmore Herbarium.

CRATÆGUS TRIFLORA Chapm. Flora Southern United States, Suppl. II. 684. 1892 [Ed. 2].—A large shrub or small tree, 2–7^{m} tall, growing on limestone bluffs near Rome, Ga. (type locality), and in similar situations near Birmingham, Ala. Main

[2] Ill. Flora Northern U. S., etc., **2**: 243. 1897.

[3] BOT. GAZ. **25**: 446. 1898.

stem single, or branching near the base into several spreading shoots, armed with numerous branched spines: bark light gray, or with a decided tinge of brown, either close or slightly furrowed and broken into many small, plate-like scales: branches ascending, clothed with light gray, smooth bark and bearing a few simple or branched, straight or slightly curved, chestnut-brown to gray spines, 2.5–6^{cm} long; they are intricately divided near the summit into many short, pilose or pubescent, brown or dark reddish-brown branchlets, forming an oblong or occasionally round or flat-topped head: buds globular, bright reddish-brown, the terminal one on strong shoots displaying several spreading scales: leaves, which are full grown at flowering time, thin at first, becoming subcoriaceous, dark green above, paler below, ovate, elliptical, or slightly obovate, acute at the apex, rounded or abruptly contracted at the base into a winged or margined petiole, 8^{mm}–3.5^{cm} long: they vary in size from those on the fertile branches, which are 2–10^{cm} long including the petiole, 1–5^{cm} wide, to large, broad leaves on vigorous shoots, 13^{cm} long, 8^{cm} wide, the borders being sharply and irregularly serrate, or doubly serrate and incisely lobed and bearing, as does the petiole, a number of stalked, black-tipped glands near the base; the upper surface harshly and rather inconspicuously pubescent throughout the vegetating season, below more densely and permanently pubescent, especially along the 5–7 prominent veins: stipules lanceolate or oblong or on the stronger shoots foliaceous and lunate, densely glandular or glandular-serrate, caducous: flowers, which open about the first of May, disposed in few-, mostly 3-flowered, pilose corymbs: pedicels 1–3.5^{cm} long, the lateral much longer than the intermediate one, bearing a densely or pectinately glandular, deciduous bractlet: calyx obconic, densely pilose, the segments persistent, pubescent, glandular-serrate, 7–9^{mm} long, 2–4^{mm} wide, acute: corolla 2–3^{cm} wide, the divisions orbicular, 10–13^{mm} in diameter: stamens normally 20, 5–7^{mm} long: styles 3–5, surrounded at the base with pale hairs: fruit, which ripens about the middle of September, globose, 12–15^{mm} in diameter, bright or deep red, pubescent, the cavity 5–6^{mm} broad, 3–5^{mm} deep: nutlets 3–5, hard and bony, 7–9^{mm} long, about 5^{mm} measured dorso-ventrally, a volume of 125^{cc} containing about 1633 clean and dry seeds.

The type material is preserved in the Chapman Herbarium at Biltmore.

Cratægus austromontana, n. sp.—A straggling shrub, 1–4^{m} in height, frequenting rocky woods and banks: main stems 1–3, arising from large coarse roots or horizontal rootstocks, or frequently a group or clump more or less united, occupying a surface of 5–10 square meters: bark close, or slightly broken into numerous small plates, gray, or with a decided tinge of brown: branches unarmed, or an occasional spine on a young plant or vigorous shoot, the bark smooth, gray or nearly brown: branchlets pilose-pubescent or tomentose, covered with dark chestnut or reddish-brown bark which is marked by round or elongated, pale lenticels, buds globular, bright reddish-brown: flowers large, borne in simple 2–5- (usually 3-) flowered cymes, opening in the vicinity of Valley Head, Ala. (type locality), the first part of May: pedicels stout, pilose or tomentose, 1–2.5^{cm} long, bearing a linear, glandular, deciduous bract near the summit: calyx broad, obconic, pubescent, the divisions lanceolate, 6–10^{mm} long, 1–3^{mm} wide, pectinately-glandular or glandular-serrate, pubescent: stamens 10, 4–7^{mm} long: pistils 3–5, surrounded at the base with pale hairs: fruit, which ripens and falls during the latter part of September, large, globose, 12–15^{mm} in diameter, bright red, pubescent, and frequently punctate, containing 3–5 hard, bony nutlets which are 8–10^{mm} long, 4–5^{mm} wide, measured from the back to the inner edge, bluntly angled on the back and with lateral faces narrow and nearly plane: cavity broad, 4–6^{mm} wide, surrounded by the persistent calyx lobes and remnants of the filaments: leaves orbicular, broadly oval or round-ovate, 3.5–12^{cm} long, including the pubescent or tomentose petiole, which, like the extreme base of the leaf, bears a number of stalked, black-tipped glands, 2.5–7.5^{cm} wide, or sometimes larger on strong shoots; they are harshly, though rather inconspicuously, pubescent on both surfaces, the pubescence being more pronounced on the lower side and along the principal veins, which are disposed in about 5–7 pairs; acute at the apex, contracted at the rounded, truncate or sometimes subcordate base into a margined or winged petiole 1–4^{cm} long, the borders very sharply and irregularly serrate, or frequently doubly serrate or incisely lobed, the serratures tipped with minute dark colored glands.

Cratægus austromontana is distributed throughout the Sand mountain region of Alabama, and has also been collected at several stations in the Cumberland mountains and hill country of eastern and middle Tennessee. The new species is closely associated with *C. triflora* Chapm., but may be recognized by its smaller size, broader leaves, fewer stamens, and by the larger and coarser seeds. The type material is preserved in the Biltmore Herbarium.

Cratægus Harbisoni, n. sp.—A tree 5–8 meters in height, frequenting rocky slopes and ridges: leaves obovate, oval, or broadly ovate, 3–12^{cm} long including the petiole, 2–9^{cm} wide, acute at the apex, narrowed at the rounded or tapering base into margined or winged petioles; they are harshly and rather inconspicuously pubescent on the upper and more densely coated on the lower surface and along the 5–7 principal veins, subcoriaceous, dark green and lustrous above, pale below, the borders doubly and irregularly serrate to near the base, or frequently incisely lobed: petioles 6^{mm}–2^{cm} long, bearing, as does the extreme base of the blade, a number of stalked, black-tipped glands: stipules glandular-serrate or pectinately-glandular, deciduous, foliaceous on the stronger shoots, acute or lunate: flowers, which appear in early May in the vicinity of Nashville, Tennessee (type locality), produced in broad, pubescent, or pilose, divergently-branched corymbs, the lower branches from the axils of leaves: bracts subtending the branches of the corymbs large, 7–18^{mm} long, 2–4^{mm} broad, pectinately or glandular-serrate, caducous: pedicels pubescent or pilose, stout, 5^{mm}–3.5^{cm} long, bearing a pectinately-glandular, elongated, deciduous bractlet: calyx obconic, pubescent, the lobes lanceolate, glandular-serrate, persistent: stamens normally 20, 4–6^{mm} long: pistils 3–5, surrounded at the base with pale hairs: fruit large, red, or orange-red, globose, 10–13^{mm} in diameter, pubescent or smooth, punctate, ripening in October: nutlets 3–5, 6–9^{mm} long, 4–5^{mm} from back to inner angle, either furrowed on the dorsal side or with a blunt ridge and two grooves, the lateral surfaces nearly plane, a volume of 125^{cc} containing about 1490 clean and dry seeds; cavity 4–6^{mm} wide: bark of the trunk, which is from 1–2^{dm} in diameter and 1–2 meters long, close or slightly fissured and scaly, ashy-gray or darker in color and frequently armed with simple or branched spines: branches clothed with smooth, gray or light brown bark, and bearing straight or curved chestnut-brown or gray spines 3–6^{cm} long, the growth of the season reddish-brown in color and marked by small, pale lenticels: buds nearly round, bright reddish-brown, the terminal one displaying several large, spreading, acute scales.

Cratægus Harbisoni was discovered by Mr. T. G. Harbison of the Biltmore Herbarium, for whom the species is named, on the limestone hills and ridges near Nashville, Tennessee, where numerous examples were observed at intervals during the past summer.

The new species probably belongs to a group of which *C. triflora* Chapm. and the *C. austromontana* previously proposed are types. From the former it may be separated by the compound, many-flowered corymbs, and from the latter by its greater size, numerous stamens, spiny branches, large flower-clusters, and the different habit of growth. The type material is preserved in the Biltmore Herbarium.

Cratægus silvicola, n. sp.—A tree, attaining in low moist woodlands a height of 6–10^{m}, or in upland forests a shrub with one or more stems, 1–5^{m} tall: trunk, which is sometimes 2^{dm} in diameter, covered with a close or slightly fissured and scaly, gray or reddish-brown bark, and armed with stout, branched spines: branches, which are spreading or ascending and form a round or flat-topped head, armed with straight or curved, chestnut-brown or gray spines 2–6^{cm} long, and clothed with gray or light brown bark: branchlets chestnut- or reddish-brown, sprinkled with small pale lenticels: buds globular, bright reddish-brown: leaves ovate, round ovate or on vigorous shoots deltoid, acute at the apex, rounded and narrowed at the base, or occasionally truncate or subcordate, 3–10^{cm} long, including the petiole, 2–6^{cm} wide, the borders sharply and irregularly serrate, or doubly serrate and incisely 5–7-lobed, the serratures minutely glandular-apiculate; they are bright or yellowish-green and minutely roughened, or occasionally scabrous-pubescent on the upper surface, paler below and generally smooth, or with a few hairs along the larger veins, which are disposed in 3–5 pairs: petioles slender, 5^{mm}–3^{cm} long, glandular: pedicels strict, 7^{mm}–1.5^{cm} long: calyx obconic, the divisions short, entire or glandular-serrate, 3–4^{mm} long, acute: stamens 10: styles 3–5, surrounded at the base with pale hairs: fruit globose, 10–11^{mm} in diameter, red or greenish-yellow with ruddy cheek, ripening and falling the last of September in the vicinity of Gadsden, Alabama (type locality): nutlets 3–5, hard and bony, 5–6^{mm} long, 3–4^{mm} measured dorso-ventrally, the back ridged and grooved and the lateral faces nearly plane: flesh thin and firm.

Cratægus silvicola is abundantly represented in the "flat-woods" of northern Alabama and northwestern Georgia, and occasionally ascends into the poorer and drier woodlands of the surrounding country. The lower leaves and those from young plants are much rougher to the touch than foliage from the upper branches of the larger and older trees, but the fruit, which when ripe falls from the trees at the slightest interference, is constant in form and color. I presume the proposed species represents one of the several forms of the South Atlantic region heretofore referred to *C. coccinea.* L.[4] Taking the original description and a copy of a tracing of the type specimen of the scarlet thorn, *C. silvicola* may be distinguished by the rough leaves, which are less incised and broader and longer in outline,

[4] Sp. Pl. 476. 1753.

and by the short, strict, and stout pedicels. The type material is preserved in the Biltmore Herbarium.

Cratægus Mohri, n. sp —A tree 6–10^{m} tall with a slender trunk 1–2^{dm} in diameter, unarmed, or sparsely furnished with long, simple or branched spines, and covered with thin, scaly, ashy-gray or reddish-brown bark; or in unfavorable situations a large, erect and branching shrub: branches ascending or nearly horizontal, spiny, forming an oblong or occasionally round compact head; the bark close and usually gray: branchlets and smaller branches zig-zag, armed with slightly curved or straight, chestnut-brown or gray spines 2–5^{cm} long; the growth of the season at first pubescent, clothed with light brown or gray, lustrous bark which is marked by small, pale lenticels: buds nearly round, or the lateral occasionally compressed, bright reddish-brown: leaves cuneate-obovate, or on vigorous shoots varying from oval to orbicular, 2–7^{cm} long including the petiole, 1.5–3^{cm} wide, or larger on the shoots, acute or rounded at the apex and contracted below into winged or margined petioles 7^{mm}–2^{cm} long, sharply and irregularly serrate to or below the middle, entire or nearly so at the base, and sometimes, especially on vigorous shoots, doubly serrate or incisely lobed; they are more or less pubescent along the veins when young, dark green and lustrous above, pale below, becoming thick and coriaceous with age: stipules linear, glandular, 5–10^{mm} long, caducous: flowers, which appear when the leaves are of full size, disposed in slender, elongated and often flexuous, many-flowered, bracteate corymbs, which are more or less pubescent or pilose at flowering time, and open in the vicinity of Rome, Georgia (type locality) about the first of May; they are 1.5–1.75^{cm} in diameter and borne on more or less pilose, slender, often flexuous pedicels 8^{mm}–2.5^{cm} long, which bear one or two minute, subulate, caducous bractlets: calyx narrow, obconic, glabrous or occasionally a little pilose; the segments linear-lanceolate, 2–5^{mm} long, entire or slightly glandular serrate, reflexed after anthesis; corolla white, the divisions round-ovate or nearly orbicular, 6–8^{mm} long, 5–7^{mm} wide with undulate or erose borders: stamens normally

20, 3–5^{mm} long: styles 3–5, surrounded at the base with pale hairs: fruit globose, 8–9^{mm} in diameter, dull, dark-red or greenish-red, or frequently covered with black spots and blotches, ripe about the first of October and hanging until early winter, the cavity 2–3^{mm} wide and deep, bordered by the remnants of the calyx lobes and stamens: nutlets 3–5, thick-walled, 5–7^{mm} long, about 3^{mm} wide measured dorso-ventrally, with a prominent ridge and two deep grooves on the back, the inner faces nearly plane.

Cratægus Mohri is distributed from Georgia westward through upper and central Alabama and Mississippi, and northward to middle Tennessee. It reaches its best development in the rich and moist soil of the "flat-woods" of central Alabama, though not infrequently it ascends into the poorer and drier soil of the hills and mountains. The species has been usually confounded with *Cratægus crus-galli* L.,[5] or more recently with *C. collina* Chapm.[6] From the former it may be recognized by the pilose corymbs, smaller and globular fruit, more numerous and smaller nutlets, habit of growth, and by the outline of the leaves; while from *C. collina* it may be separated by the later time of flowering, smaller fruit and nutlets, and by the luster of the leaves.

I take pleasure in associating with this beautiful and most distinct hawthorn, the name of Dr. Charles Mohr, of Mobile, Alabama.

The type material is preserved in the Biltmore Herbarium.

BILTMORE HERBARIUM.

[5] Sp. Pl. 476. 1753.

[6] Flora S. U. S., suppl. II. 684. 1892. [Ed. 2.]

BRIEFER ARTICLES.

SOME PECULIARITIES IN PUCCINIA TELEUTOSPORES.[1]

(WITH SIX FIGURES)

THE distinguishing characteristics of many Puccinia teleutospores are very slight, while on the other hand such species as *P. podophylli, coronata, pruni*, etc., are, in typical specimens, at once set aside from all others by the markings of their epispores. In many species, variations in the number and position of the septa are characteristic. Generally, however, the shape and size of the spores are fairly constant, and this is particularly true of *P. graminis* Pers. and allied gramineous rusts. Occasionally one-celled spores are found, due perhaps, as Professor Burrill[2] suggests, to insufficient nutrition. Eriksson and Henning[3] call attention to the so-called mesospores and other peculiarities found among spores from closed sori of *P. graminis*, attributing them to the pressure of the epidermis, and Bolley[4] in regard to the anomalous shapes found among spores says "certain it is that pressure within the crowded sorus is capable of producing an almost unlimited number of irregularities in the spore forms." J. A. Warren[5] has written regarding some very striking variations in the spores of *P. Windsoriæ* Schw., while Dietel,[6] writing on peculiarities in Puccinia spores, refers to the finding of a well-developed four-celled spore of *P. graminis*, and from time to time the odd shapes of rust spores have been noted by various writers.

Puccinia heterospora B. & C. is a species showing many interesting variations, giving as they do an indication of the close relationship of the Uromyces and Puccinia genera of the Uredineæ; in fact, this species must be regarded as one of the connecting links between the two. The spores are of two kinds, one and two-celled, the one-celled being globose to subglobose, and measuring 19 × 28μ.

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[1] Contribution from Botanical Dept. Iowa State College of Agriculture and Mechanic Arts, no. 16.

[2] Parasitic fungi of Illinois **1** : 171. 1885.

[3] Die Getreideroste 129. *pl. 4*.

[4] Sub-epidermal rusts. BOT. GAZ. **14** : 139–144. *pl. 15*. Je. 1889.

[5] Notes on the variations of *Puccinia Windsoriae*, Am. Nat. **32** : 779–781. *pl. 1*. 1898.

[6] Beiträge zur Morphologie und Biologie der Uredineen. Bot. Centralblatt **32** : 86–88. 1887.

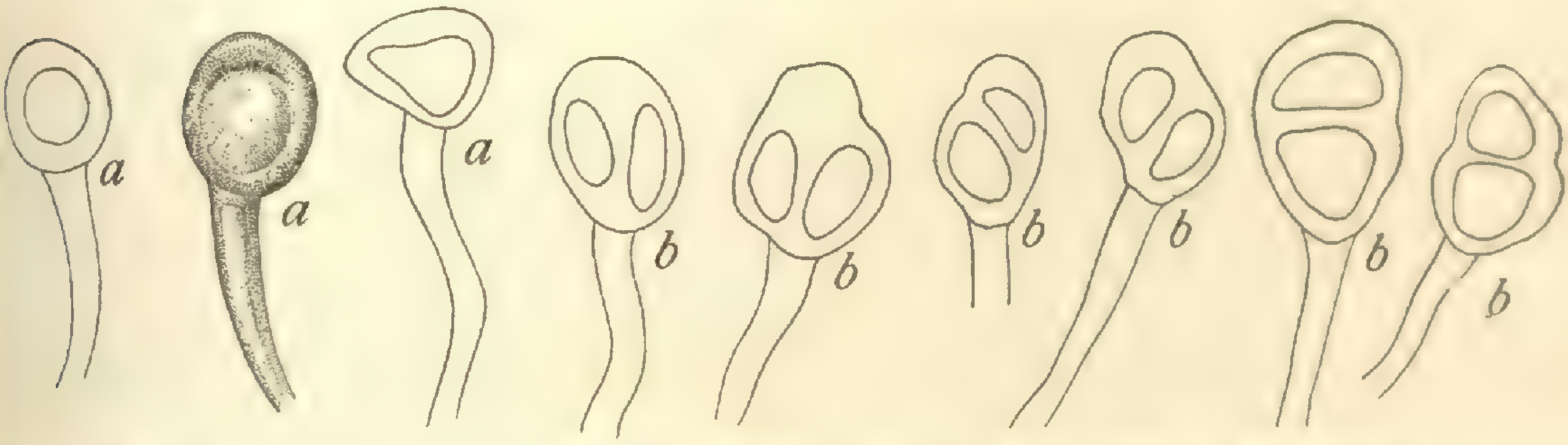

FIG. 1.—Teleutospores of *Puccinia heterospora*. *a*, one-celled; *b*, two-celled.

The two-celled spores are of three kinds: some have the septum transverse, in some it is oblique, while in others it coincides with the axis of the spore. The measurements do not differ materially from those given for the one-celled spores. The one-celled forms are much more numerous and the epispores of all are thick and smooth.

In 1884, Dr. Trelease[7] described a species occurring on *Bromus ciliatus* and called it *P. tomipara*. He says, "this species is remarkable from the fact that the spores are commonly three or four-celled with the uppermost septum oblique or not infrequently parallel to the axis of the spore, which is thus made to consist of more than one row of cells." To this comment on the very variable spores of this species nothing can be added.

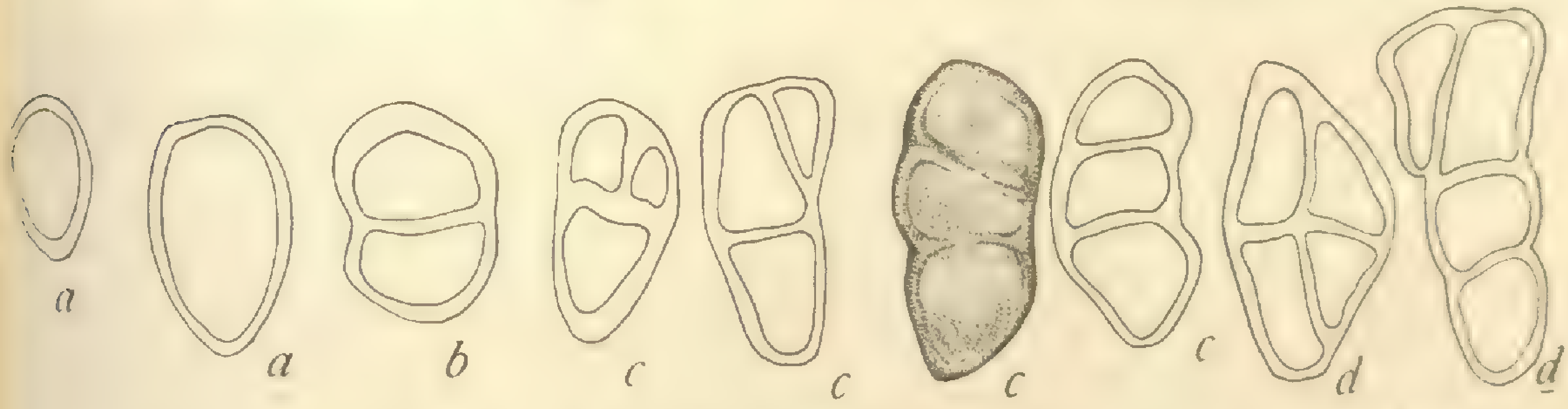

FIG. 2.—Teleutospores of *Puccinia tomipara*. *a*, one-celled; *b*, two-celled; *c*, three-celled; *d*, four-celled.

Puccinia irregularis E. & T. is another species showing variable teleutospores, one, two, and three-celled spores being found. The septa are always transverse. In addition to the variation in the number of cells, the spores are peculiar because of the position of strongly developed papillae. Usually a single papilla is found at the apex of the spore, but often the spores are truncate, when two papillae appear, one on each side. In some the papilla is found just below the septum. One spore was observed having the whole of one side thickened instead of the apex. The spores measure 50–75×19–28μ, the one-celled spores being somewhat smaller.

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[7] Preliminary list of parasitic fungi of Wisconsin 22–23. N. 1884.

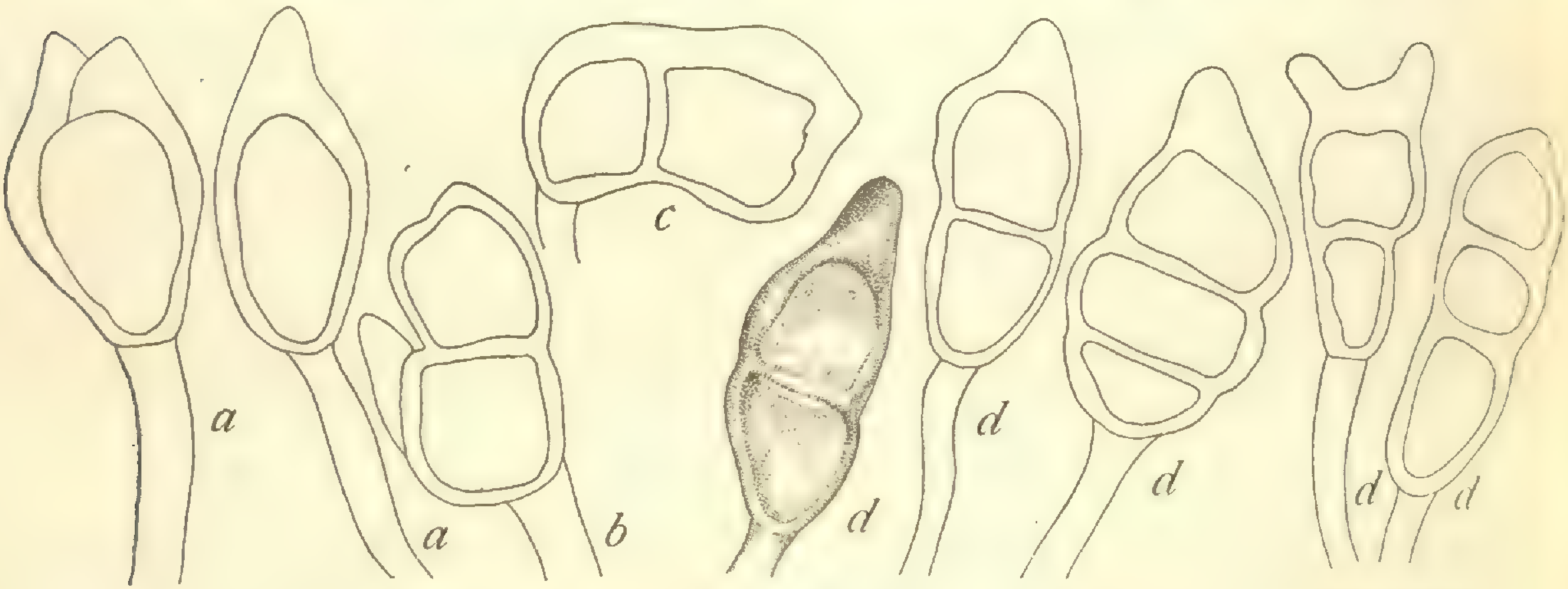

FIG. 3.—Teleutospores of *Puccinia irregularis*. *a*, one-celled; *b*, with papilla below septum; *c*, with side thickening; *d*, two- and three-celled.

An examination of the material in the herbaria of the Missouri Botanical Garden and the Iowa State College leads to the belief that this is undoubtedly the species referred to *P. Solidaginis* Pk. by Dr. Trelease, no. 169, *Preliminary list of parasitic fungi of Wisconsin*.

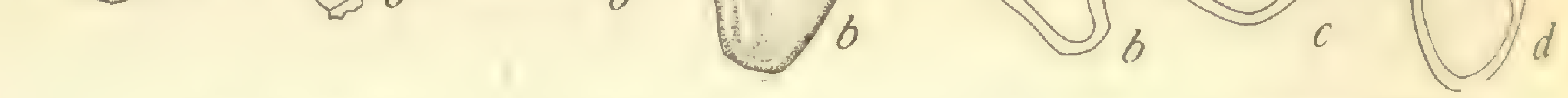

FIG. 4.—Teleutospores of *Puccinia Montanensis*. *a*, normal; *b*, three-celled; *c*, four-celled; *d*, five-celled.

A specimen of Puccinia occurring on *Elymus robustus* was collected at Ames, Iowa, by G. W. Carver, October 14, 1895. The greater number of the spores were quite normal in shape and just what might be expected in a Puccinia, but among them were found a few spores of more than two cells. The multicellular spores were in some cases not unlike those of *P. triarticulata* B. & C., but owing to their scarcity and the fact that the two-celled spores agree in size and shape with those of *P. Montanensis* Ell. it is best to regard it as that species. The position of the septa in some of the spores would be very hard to describe. Some spores were quite regularly three and four-celled, while in others oblique, transverse, and perpendicular septa were present, all in one spore. The three-celled forms were of two kinds, regular ones, and those in which two cells were found at the apex with one at the base, but occasionally this was just reversed. The measurements varied from $33 \times 25\mu$ to $55 \times 33\mu$.

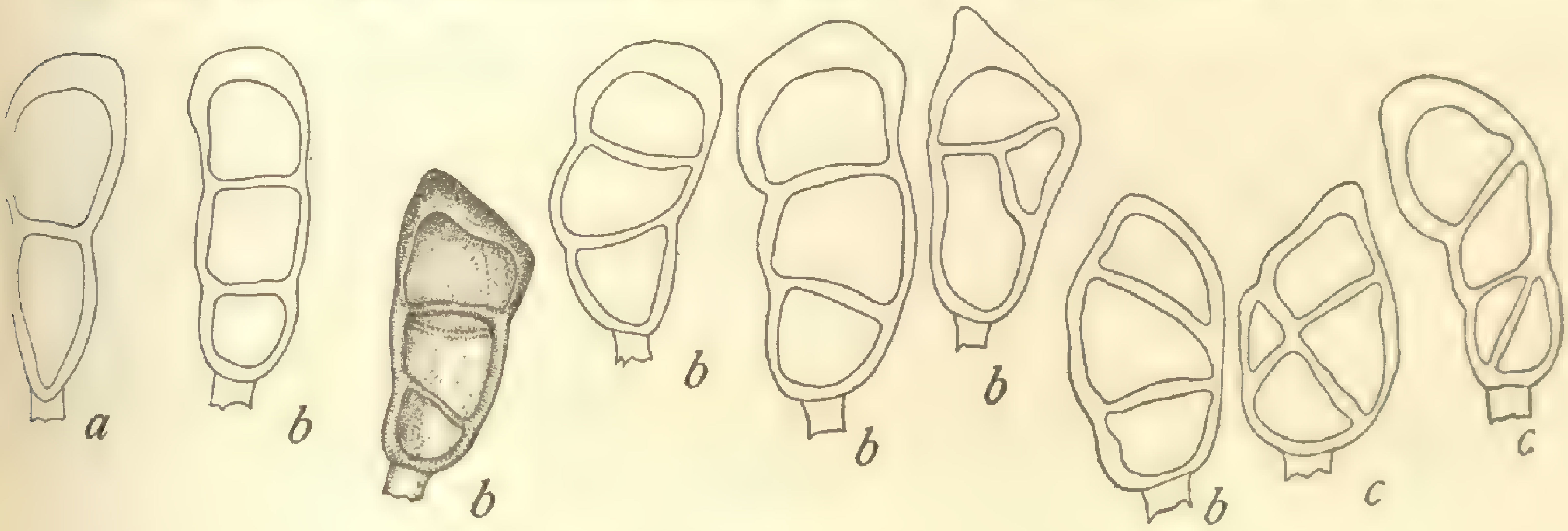

FIG. 5.—Teleutospores of *Puccinia Rubigo-vera*. *a*, normal; *b*, three-celled; c, four-celled.

A specimen of *Puccinia Rubigo-vera* (DC.) Wint. on *Agropyron tenerum*, collected at Ames in 1896, showed a considerable number of spores having three and four cells. The normal two-celled spores were greatly in excess of the others, being found in the proportion of about fifty to one. The three-celled forms were usually somewhat irregular, though occasionally one was found in which the septa crossed the spore at the right angles to the lateral walls. The four-celled forms were fewer in number than the three-celled, only a half dozen being found in three mounts. The septa were so placed as to divide the spore in different planes. The measurements of all were confined within the usual limits for this species, $13\text{–}20 \times 28\text{–}56\mu$.

On November 24, 1898, the writer picked up a piece of rust-affected oat straw (*Avena sativa* L.) on the road in front of the dairy building at the Iowa State College. The rust sori presented all the features of the sori of *P. graminis* Pers., appearing on the sheath as black linear or oval confluent patches.

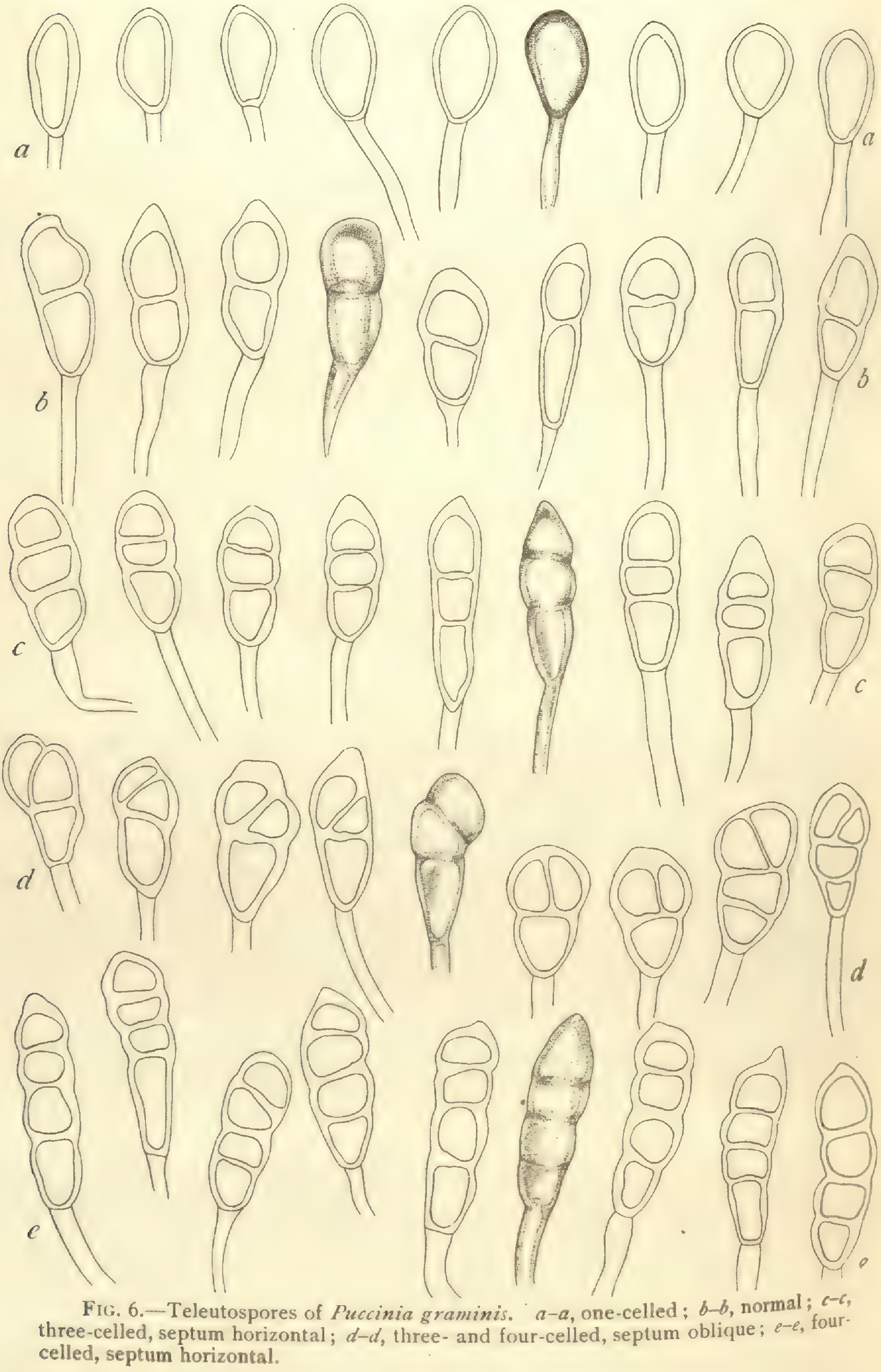

FIG. 6.—Teleutospores of *Puccinia graminis*. *a–a*, one-celled; *b–b*, normal; *c–c*, three-celled, septum horizontal; *d–d*, three- and four-celled, septum oblique; *e–e*, four-celled, septum horizontal.

A microscopical examination made sometime afterward revealed some noteworthy peculiarities in the shape, size, and number of cells in the teleutospores. The usual two-celled spores were present but accompanied by others having one, three, and four cells.

The different forms were about equal in number but differed considerably in size, the four-celled variety being the largest, as one would naturally expect. Of each form ten measurements were made, giving the following extremes: one-celled, $27–36 \times 15–20\mu$; two-celled, $30–45 \times 15–21\mu$; three-celled, $45–54 \times 15–21\mu$; four-celled, $52–66 \times 15–20\mu$.

The one-celled spores might easily have passed for the teleutospores of some Uromyces such as *U. graminicola* Burrill. The two-celled ones were quite normal in size and shape, except that in some the pedicels were much stouter than are usually found in *P. graminis*, more closely resembling the pedicels of *P. emaculata* Schw. The three and four-celled forms were of three kinds, some having the upper septum horizontal, some oblique, and others vertical, as though the upper cell had been formed as a sort of afterthought, by the division of the second or third cell as the case might be.

In these the evolutionary development of several genera of Uredinae could be plainly traced, passing from the lower Uromyces through Puccinia and Triphragmium to Phragmidium. The spores, aside from the number of cells, were not likely to be mistaken for those of Triphragmium or Phragmidium, as they were quite different in general appearance. These genera have undoubtedly a common origin, and must be looked upon as being more highly developed, more specialized, in direct relation to the number of cells in the spores, as it is quite apparent that a larger number of sporidia can be produced with less effort in those having the larger number of divisions in the spores.— H. HAROLD HUME, *Iowa State College, Ames.*

WHAT IS PRUNUS INSITITIA?

IN the June number of the BOTANICAL GAZETTE there appeared an article under the heading given above and written by Professor F. A. Waugh. The conclusion to which the author arrives, in his own words, is "that there is no such species as *Prunus insititia*."

To me this seems rather strange. I happen to have been born in the land of Linnæus and received a large portion of my botanical education at the Royal Gymnasium at Skara, Sweden. As a boy, I used to pick and eat the fruit of what there is known as *Prunus insititia*, and as a young botanist I made herbarium specimens thereof. I know that the tree which goes under that name is more distinct from *P. domestica*, as well as from *P. spinosa*, than *P. hortulana*, or *P. nigra*, or even *P. angustifolia* is from *P. Americana*. I know that there are at least three species of plums in Sweden, for I have seen them myself.

The latest catalogue of the plants of Scandinavia, published in 1897, also gives the following plums: "*Prunus spinosa* L., *P. spinosa coætanea* W. &. Gr., *P. insititia* L., *P. insititia rustica* Hn., and *P. domestica* L." Of these the first three are recognized as being natives of Sweden, while *P. domestica* and *P. insititia rustica* are regarded as only escaped from cultivation. So far as I know, *P. insititia* L. has always been regarded as a good species in Sweden; but let us see how botanists of other countries have treated it.

As Linnæus in the original description stated that *P. insititia* is a native of England and Germany, it will suffice to see how the botanists of those countries have treated the species. In almost every German flora *P. insititia* is regarded as a good species. Koch, the acknowledged authority in Germany, recognized it, and in Thomé's elaborate work there is an excellent description.

It is true that Bentham put *P. domestica*, *P. insititia*, and *P. spinosa* into *P. communis* Huds.; but Hooker, who has always been known for his conservatism, recognized all three as distinct species, not to mention other less important English botanists. It is figured in Sowerby's English Botany **12**: 841.

With these facts in view, it is surprising that one who has not studied the native plums of Europe in the field, with the few specimens found in the American herbaria, undertakes to settle the existence or nonexistence of *P. insititia*, and can state positively "that there is no such species as *P. insititia*."

If Professor Waugh had said that *P. insititia* is the same as *P. domestica Damascena*, or that *P. insititia* is not found in America, I should have been the last to criticise. I have not the means to disprove the former, and I am more than willing to accept the latter. *P. domestica Damascena* L. was based upon "*Pruna majora dulcia et parva atrocaerulea, Bauh. pin. 443*, n. 23," and *P. insititia* L. on "*Pruna sylvestria praecocia, Bauh. pin. 444*." Apparently, therefore, they seem to be two different things. For that matter they might well belong to the same species. Koch states in the older edition of his *Flora* that Linnæus included several forms in *P. domestica*, which rightfully belonged to *P. insititia*. Even this question has to be settled in Europe.

As to the non-existence of *P. insititia* in America, I agree fully with Professor Waugh, for the following reasons: If Dr. Gray had had what is known as *P. insititia* in Sweden, I doubt that he would have made it a variety of *P. spinosa*. Dr. Gray's statement that it is "adventitious in hedgerows" made me very suspicious when I saw it in his manual a year or two ago; for *P. insititia*, so far as I know, is never used for hedges. I think that *P. insititia* should be erased from the list of American plants.—P. A. RYDBERG, *New York Botanical Garden.*

NOTES ON THOREA.

(WITH PLATE XXVI)

ON October 1, 1898, Mr. A. A. Hunter, collector for the botanical department of the University of Nebraska, found specimens of *Thorea ramosissima* Bory in Rock creek, a small stream near Lincoln, Neb.[1] The plants were floating from a gravelly bottom in swift running water at a depth of half a meter and were surrounded by a mass of other algæ, principally Vaucheria. Subsequent search for Thorea in this locality has thus far proved unavailing.

So far as we know, Thorea has been found to a certainty in but three other localities in North America. E. Hall collected a specimen of *Thorea ramosissima* Bory in the Sangamon river, Illinois, in 1866, and this, with specimens of other fresh water algæ, was afterwards sent to the Botanical Museum of Berlin, where it is still preserved.[2] Francis Wolle found a mere fragment of Thorea in a lake at Winter Park, Florida, date not given.[3] Professor De Alton Saunders,[4] in December 1898, found Thorea in abundance in running water from springs in Texas, the stations being San Marcos (Hayes county), New Braunfels (Comal county), and San Antonio (Bexar county).

[1] See notice in BOT. GAZ. 27:71. 1899.

[2] MAGNUS: *Thorea ramosissima* Bory bei Belgrad in Serbien und denen weitere Verbreitung. Hedwigia 38:114. 1899.

[3] WOLLE: Fresh water algæ of the United States, 58. 1887.

[4] Communicated in a letter, accompanied by specimens in formalin.

Thorea is widely distributed over the world, having been reported from France, Germany, England, Denmark, Austria, Venezuela, Ecuador, Java, and the Marianne islands. In Ecuador it is said to be especially abundant.

Our description of the Nebraska plant does not differ essentially from that of Schmidle in his excellent monograph of *Thorea ramosissima* Bory.[5] In our plant the body consists of long cylindrical branches, originating near the base, and these again have occasional branches. The color is an olive green, rather than the black or brown color mentioned by Schmidle. When dried, the plant retains its olive green color, becoming somewhat brownish. The whole plant is about 5^{dm} long, and 2 to 3^{cm} wide, when floating in the water. Each branch consists of two distinct portions, viz., an outer covering of several-celled hairs or ramelli, and a denser axial portion of interlacing cellular filaments, which are held more firmly together by a mucilaginous matrix which sheaths every fiber and extends outwards as far as the first two or three basal cells of the hairs. The surrounding zone of hairs has a width of from 400 to 600μ, being of nearly the same diameter upon all portions of the plant body, except at the base. The axial portion has a varying diameter, ranging from 700μ at the base to less than 100μ at the growing point of the branch.

The hairs grow at right angles to the axis and constitute two quite distinct belts; an outer belt of quite evenly distributed long hairs, having an average length of 500μ; and an inner belt of clustered, short hairs of an average length of 70 to 90μ. The long and short hairs are intermingled, and both kinds spring from the same basal cell. The short hairs are protected by gelatinous sheaths, which are extensions of the central gelatinous matrix. The cells of the long hairs are rectangular in shape, and quite uniform in diameter. The short hairs have shorter cells, which are also nearly uniform in diameter, yet in some cases they taper slightly towards the apex. In the older portions of the branches the short hairs are more numerous, while in the younger region the long hairs predominate. As the plant matures, the apical cell of the short hairs often develops into an asexual spore (aplanospore). Among the short hairs, and often from the same basal cells, there may arise narrow hairs which develop a small cluster of similar asexual spores, rarely over five in number.

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[5] SCHMIDLE: Untersuchungen über *Thorea ramosissima* Bory. Hedwigia **35**:1–33. 1896.

In both cases the spores when young are spherical, and when mature the former are oval, the latter pyriform.

The axis consists of three structural parts: (1) a more or less distinct outer portion, of irregular basal cells from which the hairs originate; (2) a belt of interlacing cellular fibers most of which run longitudinally in the axis; these fibers are connected either to certain hair clusters by basal cells, or in some cases to a single long hair; there are frequent oblique or transverse fibers among the longitudinal; (3) the innermost portion of the axis consisting of an interlacing mass of cellular fibers running in all directions; these are connections or continuations of the outer longitudinal and transverse fibers.

Each hair cell contains greenish, disk-shaped chromatophores, and a distinct nucleus. There is also a distinct protoplasmic connection between the cells through the center of each cell partition. The fibers near the outer edge of the axis, especially those directly connected to the hairs by basal cells, contain chlorophyll bodies more or less irregular in shape, and show in many cases intercellular protoplasmic connections similar to those in the hairs. The intercellular walls of the internal fibers are often oblique, but are always transverse in the enlarged portions. Towards the center of the axis chlorophyll bodies become rarer, and in some fibers entirely disappear.

We found, also, in the outer portion of the axis certain longitudinal fibers, which show no chlorophyll bodies, and whose protoplasmic contents seem to be homogenous. Cell partitions in these are either lacking or at considerable distances apart. These fibers branch occasionally, and are connected in a few cases to basal cells of hair clusters. Others are united to the oblique or transverse fibers. In sections which had been treated on the slide with acid alcohol to remove the gelatinous sheaths, and first stained for two or three hours with acid orseillin, then with methyl green or echtgrün for one minute, these fibers were differentiated from the others, the cell contents staining red in contrast to the thick unstained or slightly green membrane. We are unable to assign any particular function to these fibers further than that they are a portion of the assimilative axial region of the plant. The hairs are both vegetative and reproductive in function.

According to Schmidle, Thorea has three distinct life forms or stages of growth. The first, or prothallium stage, consists of more or less branched cellular fibers which develop directly from the spores. We found plants in this stage, but found neither spores nor tetraspores developing from them. Schmidle found what he thought might be tetraspores, but questions their existence. The second, or Chantransia stage, develops directly from the former. The plants assume a Chantransia-like form, growing up in little dark green or brown tufts on the surface of stones, etc., at the bottom of running streams. In this stage Thorea bears asexual spores in abundance. The third and most highly developed form of Thorea is the branching plant body described above. It develops from a union and growth of a number of Chantransia-like plants into a branching thallus of greater size, yet possessing all the forms of structure found in the preceding stages, with the addition of carpogones.

The position of Thorea was long in doubt. Moebius in 1891–2 placed it among the Florideæ.[6] Schmitz in 1892 placed it among the Phaeophyceæ,[7] but changed his mind in 1894[8] and left it between this group and the Florideæ, giving preference to the latter. Schmidle, who has devoted more time to the study of Thorea than any other botanist, is certain that it properly belongs to the Florideæ for the following reasons: (1) in containing phycoerythrin, like the red seaweeds; (2) in having cystocarps resembling those of Batrachospermum; (3) in that the hair cells contain intercellular protoplasmic connections typical of many of the lower Florideæ; (4) in developing from Chantransia-like forms in much the same manner as Batrachospermum.

The Nebraska specimens of Thorea agree in points of general structure with the published descriptions of *Thorea ramosissima* Bory, with a few exceptions. The plants have a decided olive-green color which persists in the herbarium specimens, rather than the purple tinge of the dried specimens from Worms and Paris. Our Thorea branches very sparingly, the longer branches often attaining a length of 3^{dm} without side branches. On the contrary, the specimens from Worms and Paris are much branched, the diameter of the zone of hairs also being two or three times greater than that of the central portion while in ours the zone of hairs has nearly the same diameter as the central portion. There is a marked difference in the hairs as found in our material and that obtained by Professor Saunders in Texas. In the former both the long and short hairs are of nearly equal diameter

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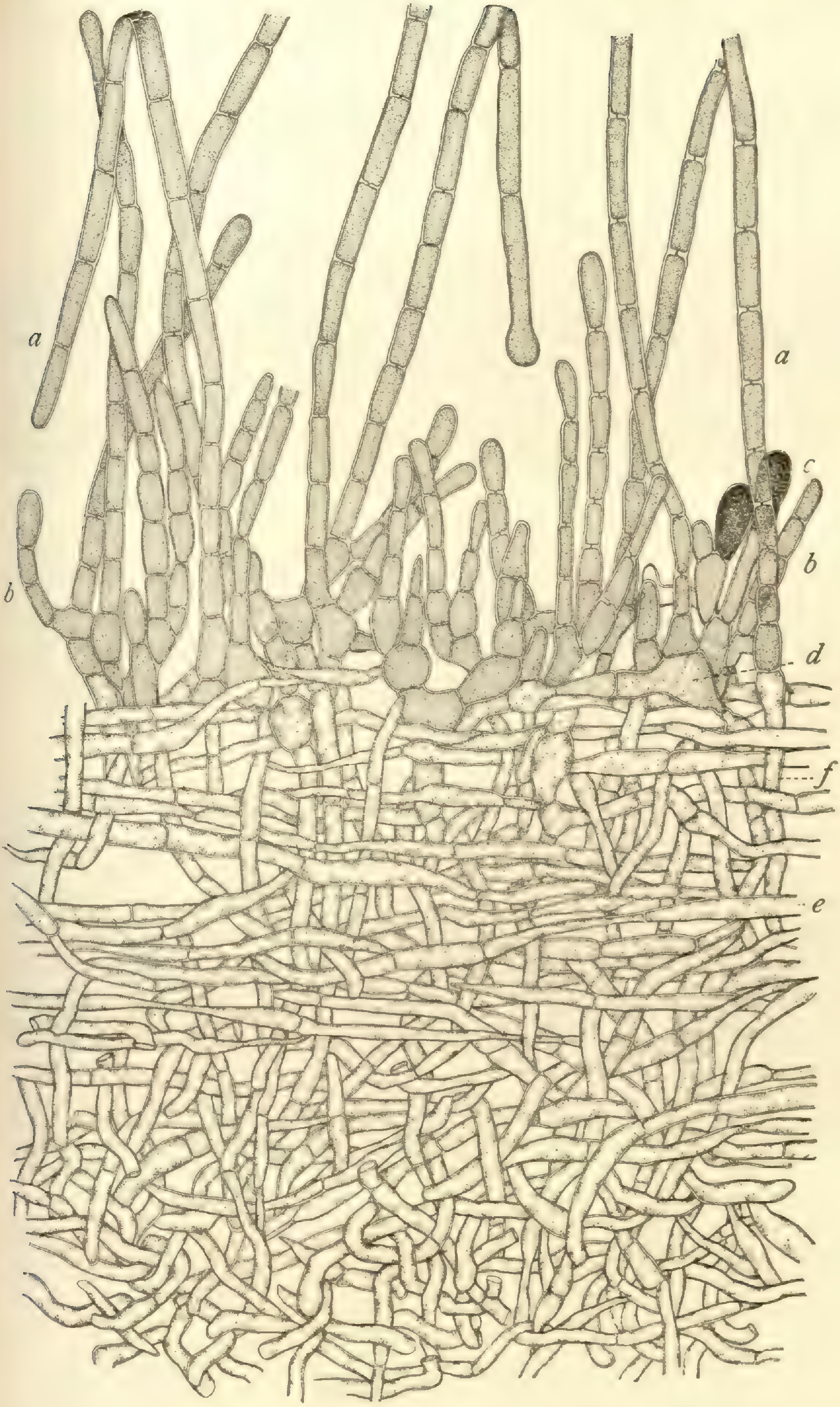
[6] MOEBIUS: Ber. d. deut. bot. Gesell. **10**: 333–344. 1891; **11**: 266–270. 1892.

[7] SCHMITZ: Ber. d. deut. bot. Gesell. **11**: 115–141. 1892.

[8] SCHMITZ: Nuova Notarisia **5**: 705–720. 1894.



HEDGCOCK and HUNTER on THOREA

throughout their length, while in the latter a great many of the longer hairs as well as many of the shorter ones taper almost to a point.

The description and measurements of structural parts are as follows:

ZONE OF HAIRS (300–500μ in diameter).

Hairs: (*a, pl. XXVI*) long hairs consisting of 18–20 (more or less) cells outside the gelatinous sheath, counting from the basal cells; diameter of outer cells 5–8μ, of inner 5–8μ; length of middle and outer (apical) cells 18–40μ, of basal 13–20μ; shape of basal cells elliptical to oval, of outer mostly rectangular, of apical usually rounded at tip.

(*b*) Short hairs consisting of 1–6 cells outside the basal cells; diameter of basal cells 6–8μ, of apical 4–6μ (rarely wider than the basal); length of cells 13-16μ; shape of basal cells round to elliptical or oval; of the others rounded to rectangular (apical often pyriform).

Spores (*c*), single or in clusters, 10–15μ in diameter, 15–26μ long, spherical when young, pyriform when mature.

INTERNAL FILAMENTS.

Basal cells (*d*) 8–18μ in diameter (broader in a few cases), 10–40μ in length (rarely larger), oblong to ovoid, often very irregular.

Cells of longitudinal fibers (*e*) 3–15μ in diameter, very variable and often indefinite in length, irregularly cylindrical and often tapering.

Cells of transverse fibers (*f*) 4–12μ in diameter, much extended and variable in length, irregularly cylindrical.

GEORGE G. HEDGCOCK and ABEL A. HUNTER, *University of Nebraska.*

EXPLANATION OF PLATE XXVI.—The plate represents a longitudinal radial section of a branch a short distance from the base, reaching from the outer edge of hairs to the middle of the axis. × 450. *a*, long hair; *b*, short hair; *c*, spore formed from a single end cell; *d*, basal cell; *e*, longitudinal fiber of central portion; *f*, transverse fiber of central portion.

NOTE ON CORN SMUT.

A FEW years ago the per cent. of smut on corn in the vicinity of Manhattan was investigated with considerable care.[1]

The tables then presented, which included three years' observations, show that the average of all counts made in August gave 6.2 per cent. of smutted stalks. This year the amount of smut was greater, one field giving 90 smutted stalks in 840, or 10.7 per cent. Another,

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[1] A. S. HITCHCOCK and J. B. S. NORTON, Bulletin 62, Kansas Experiment Station, December 1896.

250 smutted stalks in 1250, or 20 per cent. These observations were made in order to compare the results with those obtained from the third field, where 140 stalks showed 38 to be smutted, or 27 per cent. This field was an experimental plat in which a number of crossed varieties were being self-pollinated. The tassels to be used for this purpose were enclosed in sacks, but the remainder were pulled out when young. At the time the pollinating was begun, several ears were beginning to silk. These were cut off with a corn knife. In some cases the entire ear was cut off; in others it was cut above the base. After a period the ears were allowed to grow as they appeared. In this last plat 117 stalks had ears upon them of which 10 had been cut. Of the 10 cut ears 9 were smutted, or 90 per cent.; of the 107 uncut ears 5 were smutted, or 4.7 per cent. The cut ears were growing at the time of mutilation. These observations serve to show that corn smut is greatly increased by mutilation which exposes the growing issue.— A. S. HITCHCOCK, *Manhattan, Kan.*

A BOTANICAL ART GALLERY

DURING the past season the University of Minnesota has taken steps to found a photographic exhibit of the vegetation of the state, and several hundred dollars have been expended for experimental work. The results are so far gratifying that the writer feels justified in giving the outlines of the plan for the benefit of other institutions that may care to develop similar exhibits.

Considerable time was spent during the summer in securing negatives of vegetation. A photographer has been continuously employed, and about 300 8 × 10 negatives have been obtained. For the present the efforts have been limited to (*a*) plant portraits in their habitats and (*b*) ecologic groups. Many of these have been enlarged and framed. A commodious and well-lighted room has been chosen for the hanging, and at present twenty enlargements, 30 × 40, and several of smaller size, have been hung as the nucleus of the gallery. The pictures are numbered and framed in the ordinary manner, and promise to have much educational value, not only to undergraduates, but to the public generally.

I find that a picture 30 × 40 can be produced, properly framed and hung, at a minimum expense of about $17. Higher prices are, however, demanded for the best work in framing. It is important to command the services of very expert photographers and skillful enlargers to obtain proper results. A good negative can be produced at an average price of $2.50. Enlargement costs about $6, and the frame cannot be secured for less than $8.50. This price is trifling when the beauty and value of the whole exhibit is taken into account.

Among the subjects of study that I have used might be named portraits of Verbascum, Euphorbia, Helianthus, Solidago, Laciniaria, Castalia, Pteris, Quercus, Cuscuta, Pyrola, and groups of shore-lines, shade-plants, mat-plants, wand-plants, forest-floor coverings, swamp vegetation, etc. Such an exhibit when fairly extended would give a very adequate and pleasing idea of the vegetation in the region that has been selected for analysis.—CONWAY MACMILLAN, *The University of Minnesota.*

A NEW LILIUM.

Lilium Masseyi, n. sp.—Bulb 12^{mm} in diameter or less, composed of fleshy scales: stem 1.5 to 3^{dm} high, with two distant scales below: leaves linear, acute at both ends or the lower obtuse, 12 to 25^{mm} long, 2 to 4^{mm} wide, in whorls of 3 to 8, the central ones generally alternate, glaucous, the margins revolute, prominently three-veined: flowers 1 to 3, erect, 2.5 to 5^{cm} high; perianth reddish-orange, its segments spatulate, obtuse, slightly pubescent, the blade 6 to 12^{mm} wide, gradually narrowed into the claw, purple spotted below: capsule obovoid, 12 to 25^{mm} high.

High mountain meadows of North Carolina, July–August. Named in honor of Professor W. F. Massey, Horticulturist N. C. Agr. Exp. Station.—C. W. HYAMS, *Agric. Exper. Station, West Raleigh, N. C.*

CURRENT LITERATURE.

BOOK REVIEWS.

Flower ecology.

As PREVIOUSLY stated in the pages of this journal (**26**: 358), the first volume of Knuth's *Handbuch der Blütenbiologie*"[1] gives a general introduction to the subject of the pollination of flowers, with the bibliography.

The second volume gives an account of the investigations which have been made upon the European and Arctic floras. The work being professedly based upon Hermann Müller's "Befruchtung der Blumen durch Insekten," even more closely resembles the English translation by Thompson, "The Fertilization of Flowers." The resemblance here extends to the introduction of the general results of other authors and in the arrangement of the families, part 1 including the Ranunculaceæ to Compositae, and part 2, Lobeliaceae to Gnetaceæ. As an example of the treatment of the particular cases we may take that of *Malva silvestris*. There is a citation of authors, an account of the mode of pollination, accompanied by a reproduction of Müller's figures, and a combined list of visitors observed by Müller and the author. Then follows the visitors observed by Alfken, Schletterer, Schenck, Loew, Scott-Elliot, and Smith. The lists are often quite fragmentary, observed in different places, and under varying conditions, so that they will hardly form a homogeneous combination, but it is a good thing to have them collected together. The fact that the most scattered and inaccessible writings are usually the most worthless does not keep anyone from rejoicing that he does not have to waste his time hunting for them. Knuth draws upon the writings of pure entomologists. Their writings are very important for the insects they especially collect, but, as a rule, they do not notice all of the visitors. Their lists are far better than those made out by persons who make you wonder if they can tell a hive-bee or bumblebee from an Eristalis.

The first part contains 697 pages, 210 figures, and as a frontispiece a portrait of Herman Müller. For a frontispiece the second part contains a portrait of Darwin, surrounded by Severin Axell, Federico Delpino, F. Hildebrand, and Fritz Müller. This part contains another 210 figures. The volume closes with a list of the flower-visiting insects, each species being followed by a list of the plants on whose flowers it has been taken.

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[1] KNUTH, PAUL: Handbuch der Blütenbiologie unter Zugrundelegung von Hermann Müller's Werk "Die Befruchtung der Blumen durch Insekten." Band II: Die bisher in Europa und im arktischen Gebiet gemachten blüthenbiologischen Beobachtungen. Teil 2: Lobeliaceæ bis Gnetaceæ. 8vo. pp. iv + 705. *pl. 1. figs. 210.* Leipzig: Wilhelm Engelmann, 1899. *M* 18. geb. *M* 21.

Facts appear here which are quite interesting in connection with statements made on page 114 of the first volume, as well as in connection with some recent writings of mine on oligotropic bees (BOT. GAZ. **28**: 32). *Andrena florea*, mentioned on the page cited as an exclusive visitor of *Bryonia dioica*, appears in **2**: 606 as a visitor of *Bryonia alba*, also, as well as *Sisymbrium officinale*, *Stellaria media*, *Rubus fruticosus*, *Cirsium arvense*, and *Carduus nutans*. *Andrena cettii* (= *A. marginata* F.), mentioned in **1**: 114 as an exclusive visitor of *Scabiosa* (Knautia) *arvensis*, in **2**: 607 is shown to visit *S. columbaria* and *S. suaveolens*, also, as well as *Succisa pratensis*, *Onopordon acanthium*, *Leontodon autumnalis*, *Hieracium pilosella*, *Jasione montana*. *Andrena nasuta* visits *Melilotus alba* also, and *Bombus gerstaeckeri* visits three species of *Aconitum* and *Gentiana asclepiadea*. So all of the exclusive cases are shown to be merely erroneous inferences from improbable data. These lists were evidently made later, and so are not connected with the first statements.

In this work the classification, although systematic within each volume, is geographical. The second volume contains observations made in Europe, excluding all others. If a native European plant has been introduced, for example, to America, any observations made upon the plant in America should be included with the observations made in Europe. This permits a convenient comparison of its normal relations at home with its acquired relations abroad. The latter are clearly subordinate and cannot take the place of the other.

If we enter upon close criticism of the present status of flower ecology, it must be said that it is a heterogeneous mixture of data derived from native plants occupying more or less normal positions in the original flora and exposed to a more or less normal insect fauna, as well as data derived from introduced and cultivated plants which seem erroneously to be expected to reveal their ecological relations without regard to their surroundings. It seems to me that introduced plants should be treated together in a supplementary part, while garden flowers should be again separated as surrounded by conditions clearly abnormal. The objection is to the mixture, not to any kind of data.

By the mystagogues, who amuse themselves by changing names, the nomenclature is quite likely to be regarded as not sufficiently unintelligible to the unskillful. But those who have to consult the literature have to use the nomenclature which is connected with it, whether they like it or not.

This work is by far the most important source of information on the facts and literature of flower ecology, and it should be in the hands of all those who are interested in the subject. I have done enough work with bibliography to observe that too large a part of it does not show anything except that the writers were ignorant of the literature. Even their interest is shown to be fraudulent. They expect us to take an interest in their writings, when they do not care enough for the subject to read what has been written by others.

CHARLES ROBERTSON.

Propagation of mosses.

DR. CARL CORRENS has been at work for five years upon the vegetative reproduction of the true mosses. In that time he has published several preliminary papers on his researches,[2] and an important collateral paper "Ueber Scheitelwachsthum, Blattstellung, und Astanlagen des Laubmoosstämmchen" in the *Festschrift für Schwendener* (1899). These investigations are now brought together *in extenso* to form a bulky volume of almost 500 pages.[3]

The gametophyte of mosses propagates itself by many methods and by brood-bodies which arise from protonema, or rhizoid, or stem, or leaf. The sporophyte produces normally no propagative organs except the spores. The homology of the brood-bodies of Georgia (with which the researches of Correns began in 1894), Œdipodium, Drepanophyllum, and a few others (all of possible paraphysine nature) remain undetermined. The term brood-organs is restricted to those structures of which propagation is the chief or the coördinate function. The term cuttings is applied to those parts artificially separated by which propagation is possible. The brood-organs of one hundred and twenty-five species are monographically described. Forty species which can be reproduced from cuttings are also elaborately treated.

The terms applied to the brood-bodies have such resemblance in form and sound in German that they must prove objectionable, particularly in speaking. *Bruchstämmchen* or *Bruchäste* are stems or branches which are brittle throughout and may break off anywhere, or even break up into pieces, each capable of growth. *Brutäste* are brittle at base only where a separation layer is formed. *Bruchknospen* are branches brittle only beneath the apex, while *Brutknospen* are short *Brutäste*. In such a case perhaps it is unimportant whether one perceives the distinction between *Bruch* and *Brut!* In the same way we have *Bruchblätter* and *Brutblätter*. *Bulbillen* are defined as *Brutknospen* with reduced leaves, but the organs described as bulbils in *Leptobryum pyriforme* bear not the slightest trace of leaf rudiments; indeed they are elsewhere called "root tubercles."

In the general part (pp. 325–460) Dr. Correns discusses the morphology and phylogeny of the brood-bodies arising on stem, leaves, and protonema.

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[2] Berichte d. deutsch. bot. Gesells. **13**: 420. 1895; **14**: 94. 1896; **15**: 373. 1897; **16**: 22. 1898.

[3] CORRENS, CARL: Untersuchungen über die Vermehrung der Laubmoose durch Brutorgane und Stecklinge. 8vo. pp. xxiv+472. *figs. 185*. Jena: Gustav Fischer. 1899. *M* 15.

He rejects Schimper's view that the cluster of gemmæ in Georgia and Œdipodium is homologous with a male "flower," the gemmæ being sterilized antheridia; and also dismisses as improbable Brefeld's suggestion that they are sterile sporangia, like chlamydospores.

The structure and development of the brood-bodies, their separation, distribution, mode and conditions of germination, and the conditions for their formation are described. Finally the author furnishes a key to the various kinds of brood organs and the species in which they occur.

A list of the literature, which consists mainly of the systematic works referred to, the special literature being very scanty, and an inadequate index complete the work.

The interest and value of the book lie in the exhaustive treatment of a subject, presumably narrow, which has shown itself broad when thoroughly studied. It would be interesting to have a similar study of the vegetative reproduction among the Hepaticæ, and we trust Dr. Correns will include in this thorough investigation both classes of the Bryophyta.—C. R. B.

NOTES FOR STUDENTS

VÖCHTING, the author of the well-known work on Transplantation and of various papers having to do with the correlations of organs and tissues, has published the results of some extended investigations on tuberous plants.[4] It is essentially, as he terms it, a study of the vicarious organs of these plants, and is a continuation and extension of a line of work begun long ago. The power of one organ to perform the function of another in case of need has long been known, and many instances of this phenomenon have been summarized by Hertwig among animals and by Goebel and Vöchting among plants. As long ago as 1803 Knight grafted the stem of a grape vine upon a petiole, and the latter organ developed woody tissue as a result. The author himself showed in previous studies that a potato tuber may be inserted into the stem and caused to develop mechanical and conductive tissues, and that the suppression of the tubers of the artichoke is followed by a swelling of other organs to take their place. In the above cases the replacing organ is essentially the same in kind as that replaced, but Vöchting now shows that almost any organ, if properly stimulated, may become a tuber. He defines a tuber as a fleshy body used for storage, whether morphologically stem, root, or leaf—a definition that the following results obviously require.

The experiments upon vicarious organs arrange themselves into two groups: a tuber may replace a stem when put in such a position that its normal function cannot be performed, or a tuber may be developed from almost any organ if the normal formation of tubers is suppressed. One of the most plastic plants studied was *Oxalis crassicaulis*, a plant which normally resembles the potato in the development of subterranean stem tubers. A tuber placed in an erect position, partially below the soil line and partially above it, develops roots and rhizomes from the buried portions and green shoots from the aerial portions. Instead of decaying rapidly, as a tuber commonly does when it has disposed of its stock of reserve food materials, the Oxalis tuber, placed in this abnormal position, lives and grows through the entire season. The tuber is obliged to function as a stem, both in the conduction of water and the plastic foodstuffs and in giving mechanical support to the aerial organs. As a result of these new functions, the author finds a striking change in the anatomy of the tuber. Instead of the predominance of parenchymatous storage cells, there is a great increase in the area of the vascular bundles; the secondary phloem and xylem develop remarkably, and the new cells and cell fusions have a much larger cross section and more complicated structures. The strong development of bast fibers and wood cells materially adds to the mechanical strength of the tuber in its new relations. Thus the tuber has become like a normal stem in structure as in function.

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[4] Prings. Jahrb. für wiss. Bot. **34**: 1–148. 1899.

The suppression of normal tuber formation in Oxalis stimulates their development in a new position. The plant sometimes develops stolons whose ends bury in the soil and develop tubers from the terminal bud. If this bud is removed, the bud nearest the end becomes a tuber, and if all the buds are removed one of the stolon internodes swells up like a tuber. In some cases the leaves instead of the internodes become tubers, and the leaflets may remain as rudiments or may swell up like the petiole. The structure of the petiole which functions as a tuber undergoes a remarkable change. The ventral furrow is absent, the petiole being round in cross section. There is no collenchyma, or green tissue, and the bundles which are so prominent in a normal petiole remain in a rudimentary condition ; even the vessels which are present may be filled up with tyloses. The changed petiole is almost wholly made up of storage parenchyma. The starch grains here often assume the most fantastic shapes ; indeed Vöchting commonly finds that the more abnormal the organ which becomes a tuber, the more abnormal are the starch grains, indicating an intimate correlation of structures of a surprising nature.

Experiments similar to those just mentioned were performed on the potato but were less successful as a rule, showing, the author thinks, that Solanum is less plastic and that the metamorphosis of stem to tuber has gone further than in Oxalis. A root of *Dahlia variabilis* was planted somewhat emerging from the soil. The new roots, which commonly form in a cluster at the base of the stem, formed at the base of the parent root, since the base of the stem was in the light. The next year the compound root system was planted with these new roots emerging from the soil, and new roots formed at the base of this second story of roots. This process was repeated until finally four tiers of roots were developed. Each year the plant started later and later, and for a long time the leaves remained yellow, showing the difficulty the plants had in conducting materials up through the series of root tubers. The fifth year the struggle was too severe, and the plant died. These tubers did not develop buds and were hence incapable of propagating the species, but experiments showed that decapitated budless tubers can remain alive and fresh for several years. The Dahlia tubers developed a stem structure like those of Oxalis.

One of the most plastic plants employed was *Boussingaultia baselloides*, a plant with the potato type of tuber. This plant grows readily from cuttings, roots and rhizomes springing from the subterranean buds, and green shoots from the aerial buds. When cuttings are placed in the soil so that all buds are in the light, the base of the stem itself, *i. e.*, the buried internode, swells up into a tuber; if the base of the internode lies deep, the tuber is elongated, if shallow, it is shortened, showing the restricting influences of light in tuber formation. As in Dahlia, these tubers are budless and remain fresh and living for a year or two, but cannot propagate the species. The change in structure is similar to that in the Oxalis petiole which becomes a tuber. A leaf of Boussingaultia when placed in the soil gives off roots, one of which swells into a budless tuber; these tubers live and function if a shoot is grafted upon them. (Leaves of Gloxinia root similarly but develop buds and are capable of growth.) The experiments on this plant and on Oxalis seem to show a strong inclination toward tuber formation, an inclination which must be satisfied in one way if not in another. In *Thladiantha dubia*, a tuberous member of the melon family, the author succeeded in setting out a tendril and developing a starchy tuber from its base, which remained fresh long after the tendril died.

Vöchting carried on several experiments for the purpose of showing the influence of external factors, especially light and darkness, upon tuber formation. The restricting influence of light in the case of Dahlia and Boussingaultia has already been indicated. In the radish the tuber is partly root but mostly hypocotyl. Plants with all the hypocotyl and part of the root above the soil tended to develop elongated tubes, to which the root contributed half or more; in some cases all of the tuber was developed from the root. Etiolated seedlings were darkened at various points by tinfoil, and tuberous swellings appeared within the darkened areas, but always as near the top as possible. Two or three separate tubers were sometimes developed in this way on a single plant. Low temperature and drought were shown to facilitate tuber formation in Oxalis, but the internal forces are so strong that they are not effective early in the season, nor, on the other hand, will warmth and moisture greatly retard tuber formation in the fall. In this species also light is unable to prevent finally tuber formation, though it greatly retards it. The strong tendency to tuber formation which finally overcomes all obstacles is due, the author thinks, not only to the internal force which looks to the propagation of the species, but also to a demand for organic symmetry; the occasional development of potato tubers without starch, and therefore functionally impotent, appears to favor this conclusion.

The author has thus been enabled to establish upon a firmer basis than ever before his ideas as to the great plasticity of plants and the vicarious nature of their organs. Perhaps the most astounding thing of all is the power shown by a mature organ, like the tuber of Oxalis or Dahlia, to be born again, as it were, and start on a period of secondary growth. The plasticity of a young organ is well known, and perhaps not so surprising, but one would scarcely have expected to see such evidences of life and vigor in a specialized organ like a tuber.— HENRY C. COWLES.

THE ORIGIN of the cilia of the spermatozoid is very briefly but clearly traced by Belajeff[5] in *Gymnogramme sulphurea* and *Equisetum arvense.* In Gymnogramme two centrosomes (the blepharoplasts of Webber and others) make their appearance at opposite poles of the nucleus of the grandmother cell of the spermatozoid. The division of this nucleus is not accompanied by a division of the centrosome, and consequently each of the resulting cells receives only a single centrosome. The centrosome, originally spherical, elongates into a narrow band lying alongside the nucleus, and the cilia arise from the peripheral portion of the band.

The sequence is the same in Equisetum, but here the writer was able to show that the band is made up of a row of intensely staining granules and a less deeply staining portion. Each granule gives rise to a single cilium.

The spherical organs which give rise to the band are regarded as genuine centrosomes, and Belajeff would homologize with them the blepharoplasts of Webber (Zamia) and Shaw (Marsilea and Onoclea), and also with the cilia-forming centrosomes of Hirase (Gingko) and Ikeno (Cycas). He would also homologize the cilia-forming band with the "middle piece" of the animal spermatozoon, as described by Hermann for the salamander. — CHARLES J. CHAMBERLAIN.

IN A STUDY of the influence of weather and the condition of the soil upon the anatomical structure of plants, W. Meyer[6] objects to culture experiments and goes for his material to nature, where plants may be found under the same conditions for many generations. He compares numerous members of the Caryophyllaceæ, chiefly alpine forms, and shows how species in different divisions of the same family have a close resemblance to one another when growing in similar situations. For example, species of the Sileneæ, Alsineæ, and Paronychieæ growing in deserts resemble each other and also those on alpine heights, for in high altitudes the sun's rays are very powerful and plants need the same protection as in deserts. On the other hand, specimens of the same species, under various conditions, show extreme divergence. He also shows that many species of the Primulaceæ resemble those of the Caryophyllaceæ when grown under like conditions. Only a causal dependence between situation and structure can explain such resemblance, since common origin cannot do it. — L. M. SNOW.

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[5] Ueber die Cilienbildner in den spermatogenen Zellen. Ber. d. deutsch. bot. Gesell **16**:140–144. *pl.* 7. 1898.

[6] Bot. Centralb. **79**: 337–350. 1899.

SEEDS whose viability had been previously tested by samples were recently submitted by Professor Dewar to the intense cold of liquid hydrogen, *i. e.*, —250° C. for half an hour. Some of the seeds were cooled in a sealed glass tube, and others were immersed without protection in the liquid hydrogen. All the seeds in both sets germinated. — C. R. B.

THE LITERATURE of diatoms has recently been enriched by a very important contribution.[7] The work is not merely a guide for the determination of the species of a limited locality, but is a comprehensive text-book of diatom lore. The author has departed from the usual comparatively superficial methods, and has taken into account the form and structure of the protoplast, the position of the nucleus, the number, form, and position of the chromatophores, the occurrence of pyrenoids, and, finally — a most important consideration — the complete life history of each species as far as this has been possible. A study of cell characters convinced the author that the number and position of chromatophores is the most important taxonomic character, and that mere frustule characters are not sufficient for determining the limits of species.

The second part of the work gives a somewhat extended account of the diatom cell, cell division, movements of diatoms, the relation of variety of form to environmental factors, the auxospores, and the rôle of diatoms in the economy of nature. — CHARLES J. CHAMBERLAIN.

THE PRODUCTION of apospory by environment has been brought about in various ferns. Mr. F. W. Stansfield[8] has succeeded in producing apospory in *Athyrium filix-fœmina, unco-glomeratum*, an apparently barren form. In all cases it was noted that prothalli are produced with much more ease from young fronds than from adult ones. If the first fronds from a prothallus are pinned down, the edges rapidly develop into prothalli. The aposporous production of prothalli is regarded as an atavistic trait, and the suggestion is made that apospory could be produced in many ferns by taking sufficient care. The fact that Mr. Druery, a few years ago, succeeded in producing apospory in *Scolopendrium vulgare*, presumably a most unlikely form for such an experiment on account of the smooth strap-shaped leaves, indicates that the suggestion has some weight.—CHARLES J. CHAMBERLAIN.

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[7] KARSTEN, GEORGE; Die Diatomeen von Kieler Bucht. Wissenschaftliche Meeresuntersuchungen herausgegeben von der Commission zur Untersuchung der deutschen Meere in Kiel und der biologischen Anstalt auf Helgoland. Abtheilung Kiel. Neue Folge **4**: 19–295. *figs. 219.* 1899.

[8] Jour. Linn. Soc. Bot. **34**: 262–268. 1899.

APPLE CANKER, which attacks the bark of the limbs of apple trees of all ages, has been traced by Mr. W. Paddock,[9] of Geneva, N. Y., to the well-known *Sphæropsis malorum* Pk., causing the black rot of apples. Cultures have been made on sterilized bean stems, and the disease produced by inoculation. In a later communication[10] further observations are given upon the destructiveness of the disease, which occurs, as it is discovered, in pears and quinces as well as in apples. Trees may be entirely killed by this disease, which in most cases progresses from the smaller branches toward the trunk.—J. C. A.

WEEDS have been the subject of a number of bulletins from the agricultural experiment stations, not yet mentioned in these pages. Only the western states are represented. F. H. Hillman (Nev. no. 38: 1–131. 127 cuts in text) describes the seeds of many weeds with much clearness and detail, and presents one hundred and twenty five cuts, drawn by himself, illustrating as many kinds of seeds. These illustrations are worthy of special commendation for their accuracy and artistic merit, and also because they are well printed. L. F. Henderson (Idaho no. 14: 91–136. 13 pl. and 5 cuts in text) discusses twelve of the state's worst weeds, and says good things about the value and justice of weed laws. E. E. Bogue (Oklahoma no. 41: 1–12. 14 cuts in text) presents information regarding seventeen weeds, of which those least known eastward are *Solanum Torreyi*, *Acacia filiculoides* and *Croton Texensis*. A. S. Hitchcock and G. L. Clothier have issued a press bulletin (no. 18) of two pages giving notes on weeds, and also a sixth report on Kansas weeds (Kans. no. 80: 113–164). A large fund of information is presented regarding the habits and distribution of weeds, not only of Kansas, but of the whole United States. Charts are used to show the distribution by counties in Kansas of 209 species, and by states in the whole country of 194 species. L. H. Pammel presents a full account (Iowa no. 38: 7–24. 7 cuts in text) of the Russian thistle, with a bibliography; also a discussion of the weeds of cornfields (Iowa no. 39: 27–52. 17 cuts in text), and of horse nettle (*Solanum Carolinense*), *Convolvulus arvensis* and *Tribulus terrestris* (Iowa, no. 42: 130–140. 5 cuts in text), the last species having recently gained a foothold on Muscatine island in the Mississippi river. E. S. Goff (Wis. no. 76: 1–53. 39 cuts in text) gives illustrations and information regarding the ten weeds mentioned in the Wisconsin weed law, with notes on eight others.—J. C. A.

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[9] *Science* **8**: 595.

[10] *Science* **8**: 836.

THE RUST FLORA of California, according to E. W. D. Holway in the October *Erythea*, embraces 122 species of Puccinia, 42 of Uromyces, and 73 of other genera.—J. C. A.

ANOTHER ARTICLE[11] has recently been added to the valuable series of physiological papers already so auspiciously inaugurated by Dr. Klebs. The same ingenious accurate experimentation which characterized the earlier papers of the series is evident. The presentation is masterly. The purpose of the research is to determine the chemical and physical factors which incite or alter the various modes of reproduction in *Saprolegnia mixta*. It is found that this species will grow indefinitely without either sexual or asexual reproduction if nourishment be abundant; but at any time the extensive formation of zoospores can be incited by simply starving the hyphæ, *e. g.*, by placing them in water. By noting the maximum concentration at which various foods induce the formation of zoospores an idea was obtained as to their relative food value. Albumens are rich; amido-acids can furnish C as well as N; in general the food value rises with the carbon content; glucosides vary from toxic to indifferent or even favorable; inorganic acids and their salts are of but little value.

By varying the nutritive value of any medium the fungus can be made at will to assume a purely vegetative condition; to produce rudimentary sporangia; to form sporangia which bear zoospores that do not escape; and to produce functional zoospores. All of these phenomena depend for their existence upon the concentration of the medium, not upon the total quantity of nutriment.

It happens, however, that even in strong solutions the formation of zoospores is often eventually suppressed. This led Dr. Klebs to infer the presence of an inhibiting agent formed in the medium by the growth of the fungus. One such substance he finds is ammonium carbonate. If the medium be rendered weakly acid zoospore formation can be resumed. Starvation, if very gradual, causes the mycelium to become too weak to build zoospores. Poisons inhibit their formation as does also high osmotic pressure. Experiments show very clearly what are the necessary relations and also the responses, but the reasons for both are totally obscure. Zoospores are never found unless the tips of the hyphæ are in contact with water. Oxygen, light, and heat are of little importance.

If a well nourished mycelium be placed in a poor medium where the conditions render the formation of zoospores impossible, *e. g.*, in a solid medium, sexual organs will soon appear in abundance. These, however, are sensitive to heat (their maximum being 26°, that of sporangia 32°) and fastidious as to their inorganic food, seeming quite dependent upon the presence of some form of potassium phosphate. This is particularly true of antheridia, and by varying the medium a filament may be obtained which bears no sex organs, or one bearing only oogonia, or one with oogonia and a few antheridia, or, finally, one with many antheridia some of which form fertilizing tubes. In this connection it should be recalled that specific distinctions have been based on the abundance of antheridia. In general the relation between oogonia and antheridia is such that support is given to the view of DeBary, viz., that the presence of oogonia induces the formation of antheridia. Dr. Klebs thinks this is due either to chemotaxis when proper inorganic salts are present, or that these salts render the twigs sensitive to the chemical stimulus emanating from the oogonia. It is evident, however, that normal oogonia can exist without inducing antheridial formation.

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[11] Zur Physiologie der Fortpflanzung einiger Pilze: Jahr. f. wiss. Bot. **33**: 71. 1899. Reviews of earlier papers may be found in this journal **23**: 214. 1893, and **27**: 77. 1899.

While as conclusively proved in this research, there is no dominating inherent tendency toward an alternation of generations, nevertheless the conditions are such that in nature an alternation is usually brought about through the exhaustion of the nutriment afforded by each newly attacked host.

Previous to oosphere formation the incipient oogonium may revert to a vegetative condition, but after the oospheres are differentiated the power to vegetate is irretrievably lost. This, the author thinks, is due to nuclear changes possibly to a chromosome reduction.

An interesting chapter is given to the consideration of gemmæ and the author concludes, apparently with ample ground, that they are of no significance in phylogeny. They are special structures whose function is to tide over times when the formation of other spores is precluded. They behave in general as do hyphæ, and develop into oogonia or sporangia according to environment. Dr. Klebs closes by saying that an acquaintance with mere morphological marks does not constitute sufficient knowledge of a species. To meet his high ideal the systematist must hereafter determine, both quantitatively and qualitatively, the life relations of the plant, its limits of variation, and the stimuli that cause these variations.— F. L. STEVENS.

WHETHER THE Saprolegniaceæ are exclusively apogamous or not is a question that has been argued *pro* and *con* in pre-cytological days by DeBary, Pringsheim, Cornu, Zopf, Ward, Humphrey, and others. Four years ago Messrs. Hartog and Trow almost simultaneously published papers expressing quite opposite views regarding fertilization in this group. Trow has recently made extended researches on Achlya[12] and arrives at conclusions in harmony with his earlier paper. He describes a karyokinetic division of the oogonial nuclei and a degeneration or digestion of the supernumerary ones, so that only one remains to function in each oosphere. Trow is convinced that true fertilization, a fusion of sexual nuclei, does occur. It is to be regretted, however, that his technique was not improved to such a point of efficiency as to insure more unequivocal evidence than he presents. The final impression that is left with the critical reader is that Trow has seen some things which make a fertilization seem possible, or even probable ; but that it is far from being proved. A really valuable feature of Trow's work consists in the observations on live material, by which he has followed the growth of the organism from the zoospore to complete maturity, including development, ripening, and germination of the oospores.

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[12] Trow, A. H.: Observations on the biology and cytology of a new variety of *Achlya Americana*. *Ann. Bot.* 13: 131.

An article which bristles with caustic but mainly petty criticism regarding Trow's conclusions and theories appears in the September *Annals of Botany*.[13] This criticism, like Hartog's criticism of Trow's earlier paper, while it increases the literature by several pages, sheds no light on the perplexing questions.— F. L. STEVENS.

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[13] HARTOG, MARCUS: The alleged fertilization in the Saprolegniaceæ. *Ann. Bot.* **13**: 447.

NEWS.

Mr. Albert H. Trow has been granted the degree D.Sc. by the University of London.

Professor Ernst Ebermayer has resigned the professorship of forestry in the University of Munich.

Mr. W. L. Jepson has been promoted from the instructorship to be associate professor of botany in the University of California.

Mr. R. K. Beattie has been appointed instructor in botany in the Agricultural College of Washington, located at Pullman.

Dr. John M. Coulter will be away from his post at the University of Chicago for nine months. He is spending the winter at Washington, D. C.

Mr. Grant Allen, author of several popular books and papers on botanical subjects, and more lately widely known as a writer of acknowledged fiction, died October 25, in his fifty-first year.

Mr. H. H. Hume, assistant in the departments of botany and horticulture in the Iowa Agricultural College, has been elected horticulturist and botanist of the Florida Agricultural College at Lake City.

The extraordinary delay in the publication of the October number was due to the illness of the present managing editor from October 13 to November 4, and the absence of the senior editor.

Two scholarships for garden pupils in the Missouri Botanical Garden will be awarded by the director, Dr. Wm. Trelease, before April first next. Applications must be in his hands not later than March first.

Mr. A. A. Heller, of Lancaster, Pa., will start for Porto Rico on a collecting trip about January 1. He expects to bring back a large and interesting collection of all classes of plants. The cryptogams of that island should be an especially interesting study.

Professor Dr. Paul Knuth, whose recent return from a tour around the world, including a somewhat prolonged stay in this country, we lately chronicled, died in Kiel on October 30, at the age of forty-five. His death will be felt as a severe loss to ecology.

Mr. J. W. Duvel, of the Agricultural Experiment Station at Wooster, Ohio, has been appointed to the D. M. Ferry Botanical Fellowship in the

University of Michigan. Mr. Duvel has begun an investigation of certain factors affecting the vitality and germination of seeds.

PROFESSOR IGNATIUS URBAN announces that the second fascicle of his Porto Rico plants is now ready for distribution, the price per hundred being *M* 40. A few sets of the first fascicle may still be obtained, at *M* 30 per hundred. Professor Urban's address is Grunewald-strasse 6/7 Berlin W.

PROFESSOR JEAN BAPTISTE CARNOY, professor of botany in the Catholic University of Louvain, died on September 8, at the age of sixty-three. Professor Carnoy was the founder and editor of the elegant periodical *La Cellule*, and the author of several works and papers of high value on cytology.

THERE HAS come into the possession of the city of Philadelphia the dwelling and part of the grounds which belonged to James Logan (1674–1751), with Penn one of the founders of Pennsylvania, and a botanist of note, after whom *Logania*, the type of the Loganiaceæ, was named by Robert Brown. The property will be known as Stenton Park, the original name of the Logan estate, as there is already a Logan Square in the city. Mr. Thomas Meehan, the venerable horticulturist, has been the mover in the proceedings which have secured the preservation of the grounds of Bartram, McMahon, and Logan.

MR. J. B. ELLIS being unable to continue the issue of *Fungi Columbiani*, has requested Mr. C. L. Shear, of Takoma Park, D. C., to take charge of it. Mr. Shear has acceded to this request, and seeks the cooperation of working mycologists and collectors. Mr. Ellis will assist in the determination of material, and doubtful specimens of the various orders will be submitted to other specialists. The series of North American Fungi has been discontinued, but the present series is in great part a continuation of it. Century XV is now in course of preparation. Complete sets of the first fourteen centuries can still be supplied.

ON JANUARY 1 the Cambridge Botanical Supply Company will discontinue the publication of the card index of American Botany, and the committee appointed by Section G of the A. A. A. S. is seeking to continue it under other arrangements. The present subscription price of $5 per year was made when only about 500 cards were issued and is inadequate to support the enterprise with the increased number of titles. It has, therefore, been decided to make the rate one cent per card. The number of subscribers will govern the number of sets printed and the matter will not be electrotyped. Intending subscribers should at once notify Professor L. M. Underwood, Columbia University, New York city.

MR. MATURIN L. DELAFIELD, JR., 56 Liberty street, New York city, having fulfilled the requirement of the constitution of the Botanical Soceity of America, becomes a patron of that organization. At the Columbus (O.) meeting last August, among other amendments to the constitution of the society, one was adopted admitting patrons. The clause relating to patrons reads as follows:

> The payment to the treasurer of the sum not less than $250 at any one time, or a bequest of such sum, shall constitute the donor a patron of the society. The names of patrons shall be published with the annual lists of officers and members, and the patrons shall be entitled to receive copies of all the publications of the society. Patrons' fees shall be added to the permanent fund of the society.

Mr. Delafield, therefore, becomes the first patron of the Botanical Society of America.— GEORGE F. ATKINSON, *Secretary*.

THE MISSOURI BOTANICAL GARDEN has this summer received a decision from the Supreme Court of Missouri which empowers the trustees of the garden at their discretion to sell unproductive endowment real estate, which was made inalienable by the will of Henry Shaw, the founder of the garden. While it is not probable that sales will be made rapidly, the purpose being to effect them at full market value, the power to dispose of this property promises to add many thousand dollars to the annual revenue of the establishment within a few years, and it makes available for immediate use each year some ten or fifteen thousand dollars which business wisdom has compelled the trustees to hold out of the current income thus far, as an emergency fund for the protection of the property when street improvements and other special expenses should be forced upon it.

Immediate use is to be made of some of the money thus liberated, by the addition to the garden of about twenty acres of ground, to be graded and planted in accordance with plans made by the landscape architects, F. L. and J. C. Olmsted, and to represent in synopsis the principal features of the North American flora.

GENERAL INDEX.

The most important classified entries will be found under Contributors, Cytology, Diseases, Ecology, Hosts, Personals, Physiology, and Reviews. New names, and names of new genera, species, and varieties, are printed in **bold-face type**; synonyms in *italics*.

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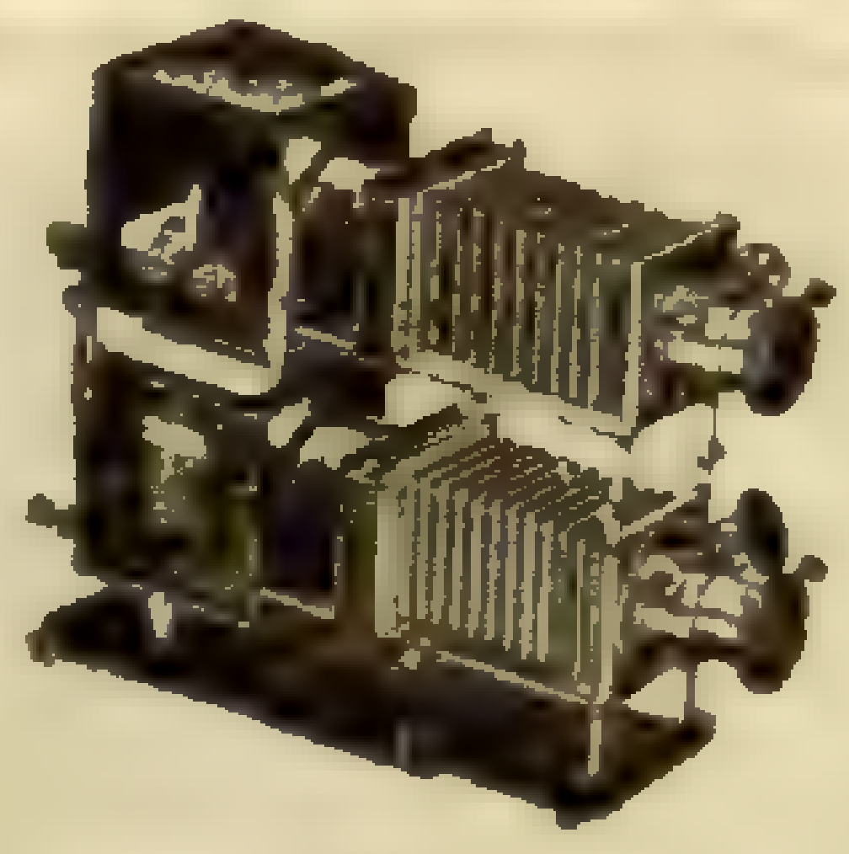

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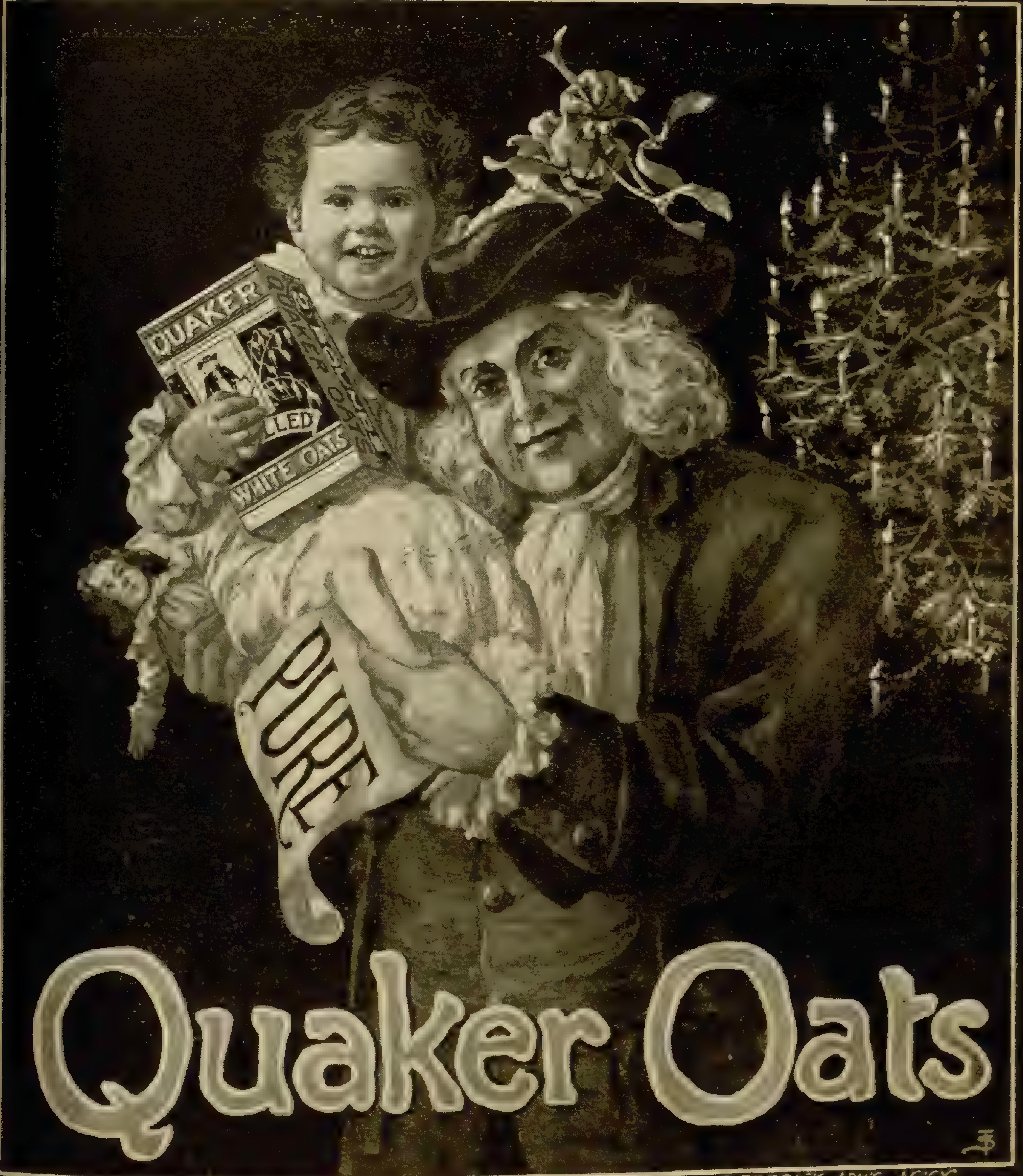

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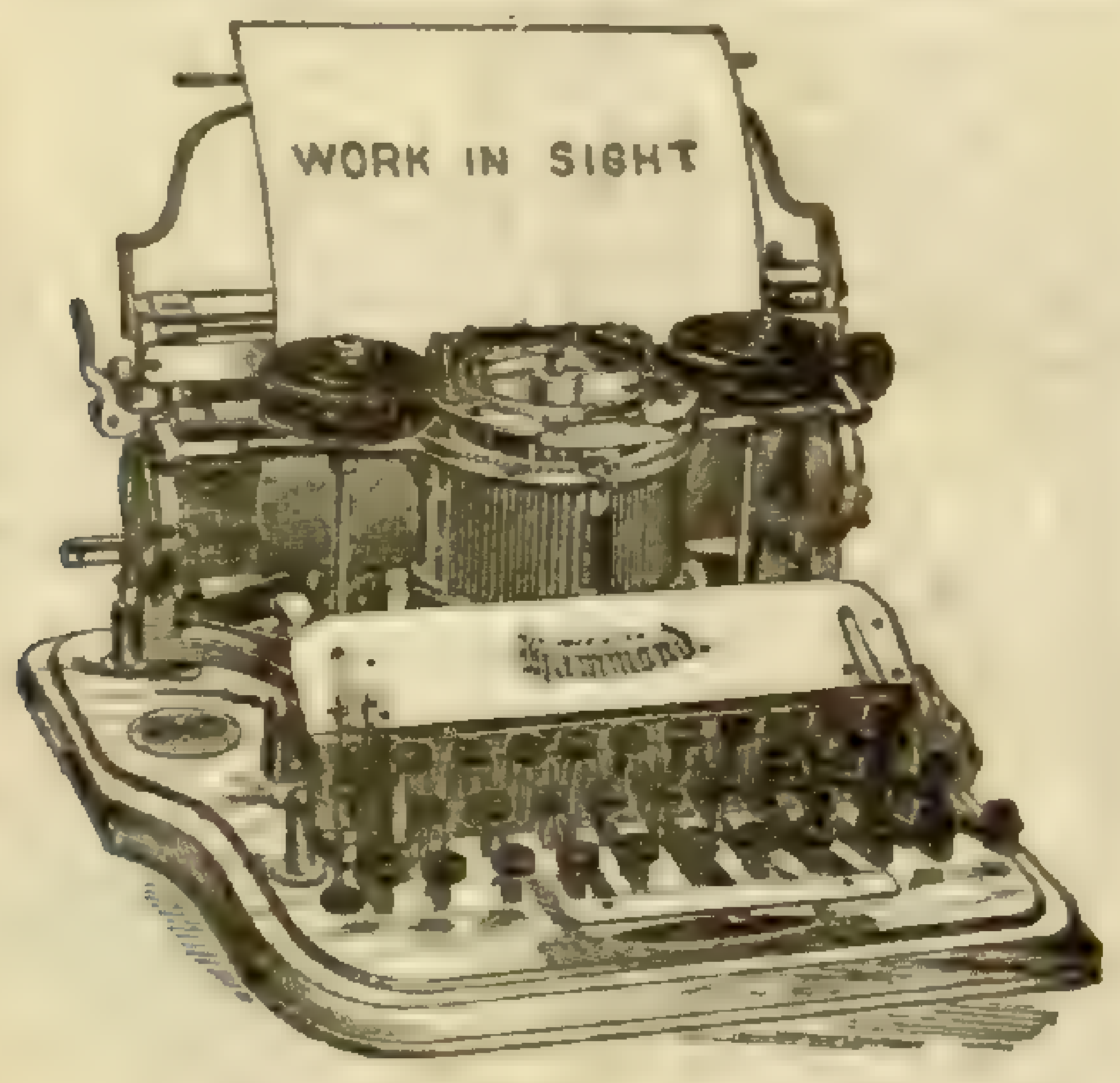

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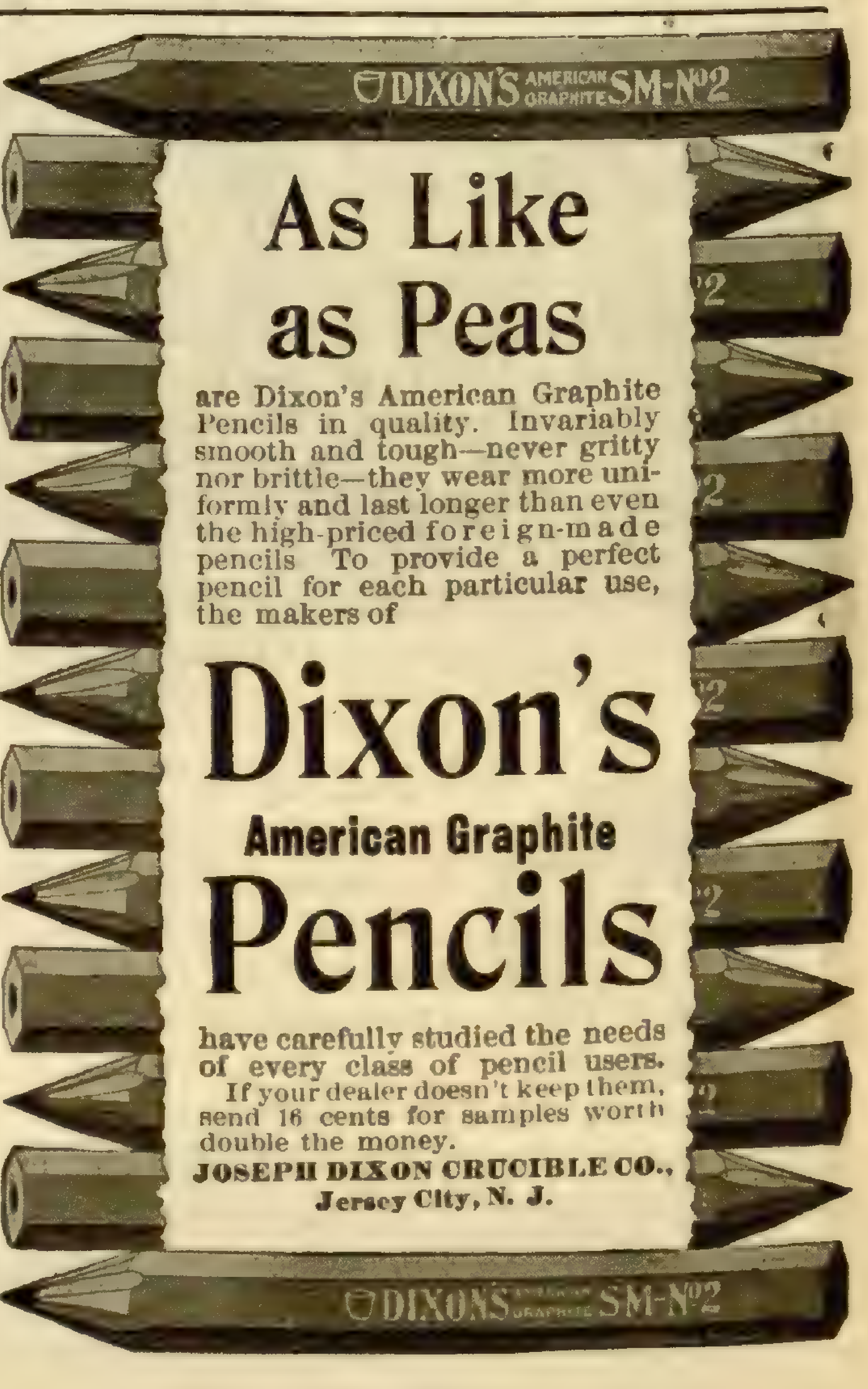
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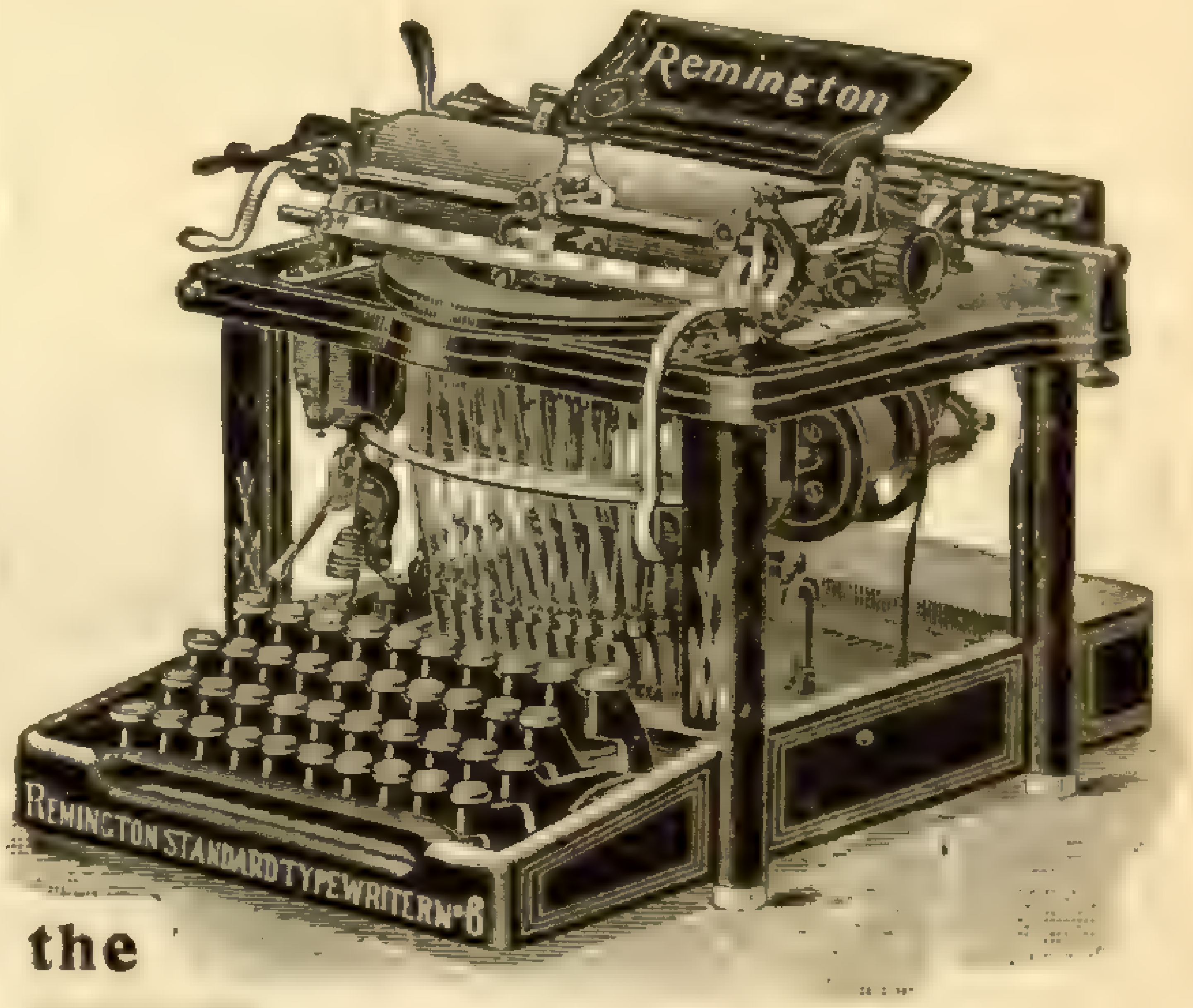

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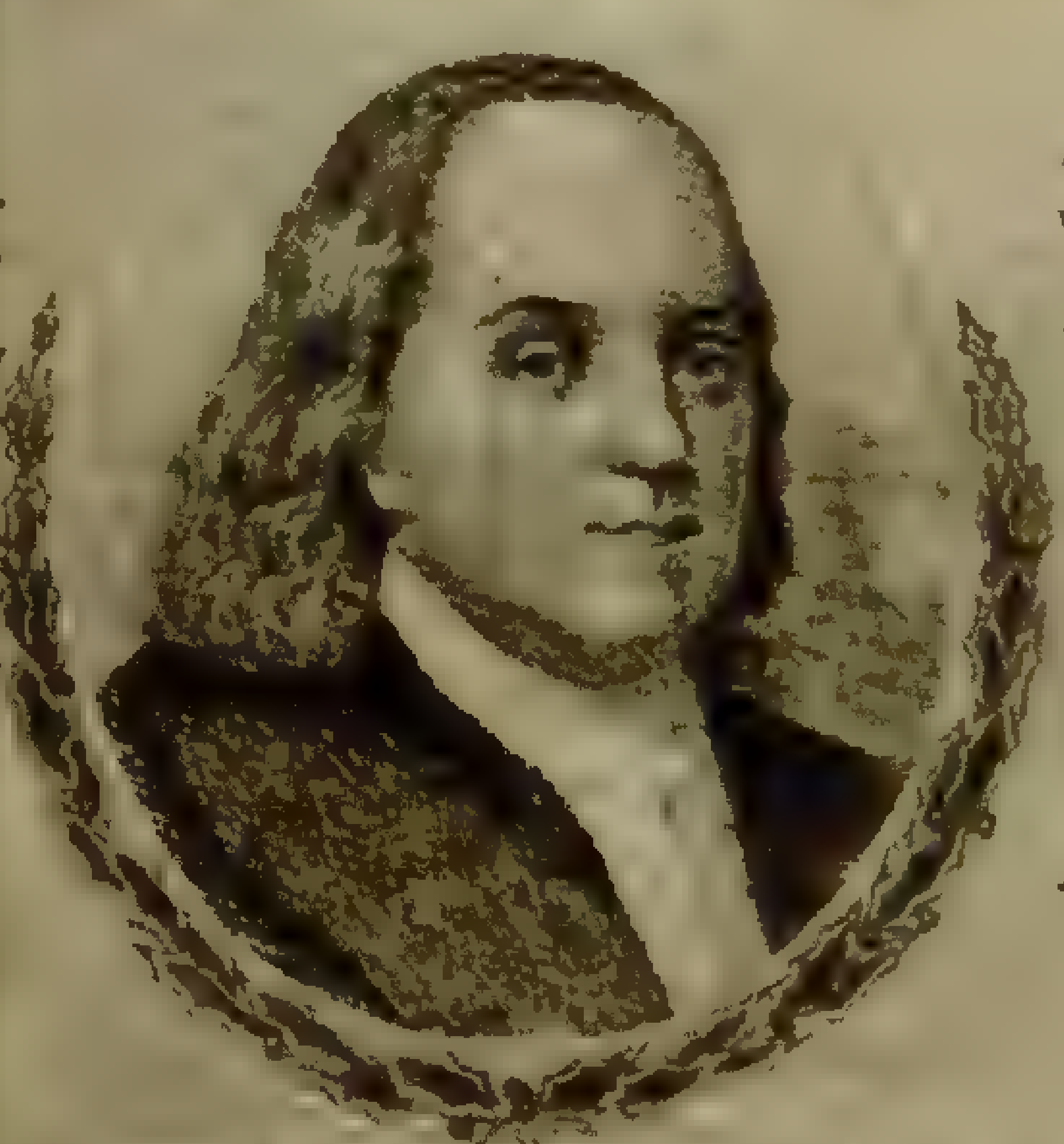

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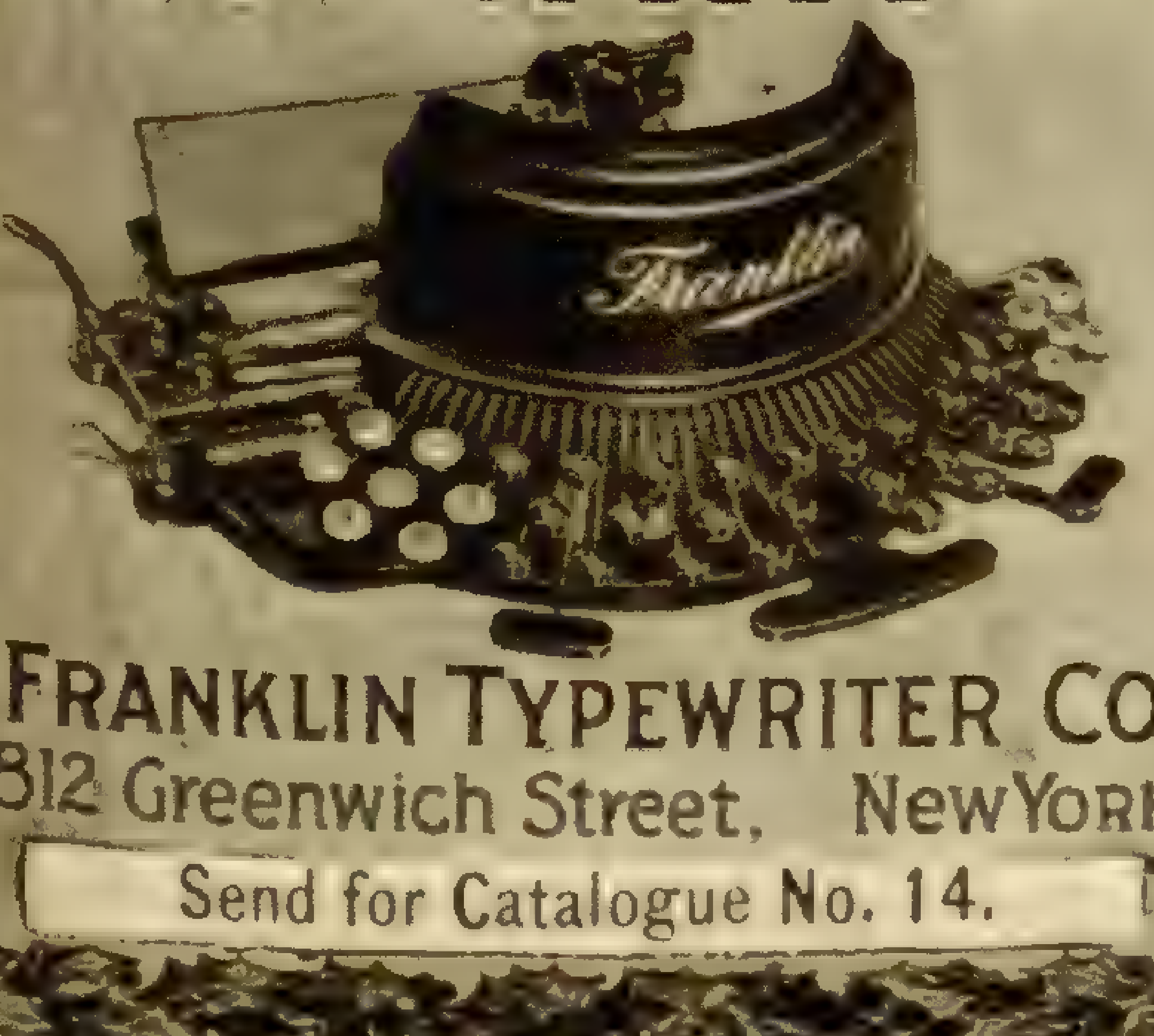

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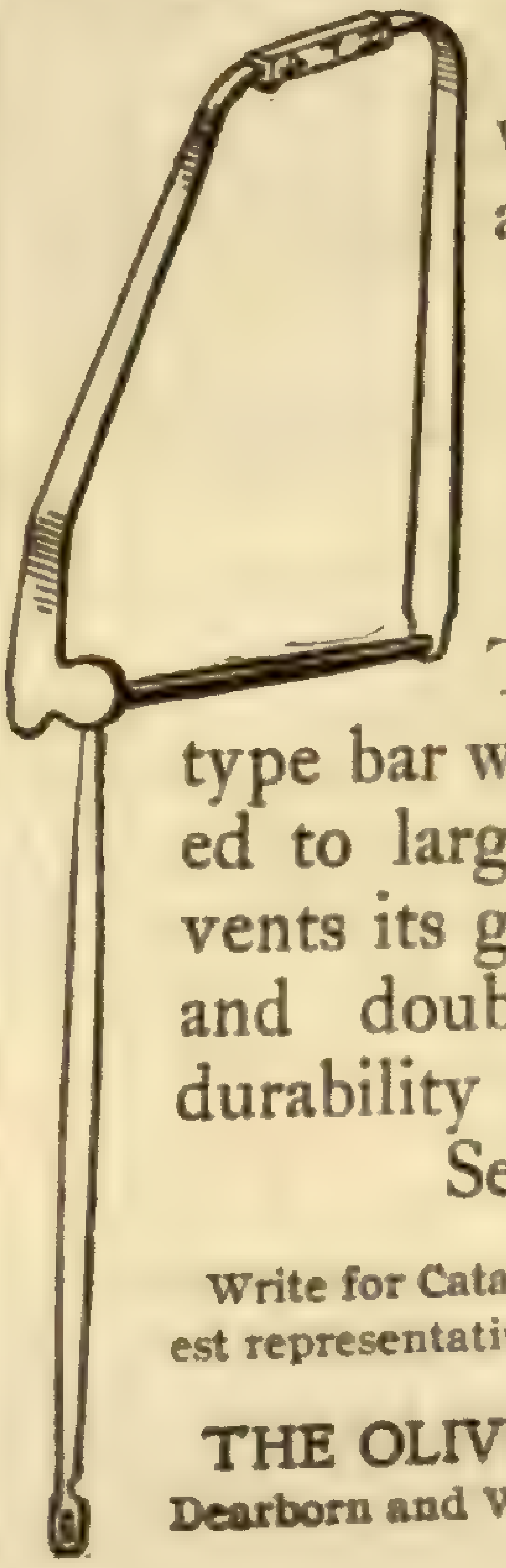

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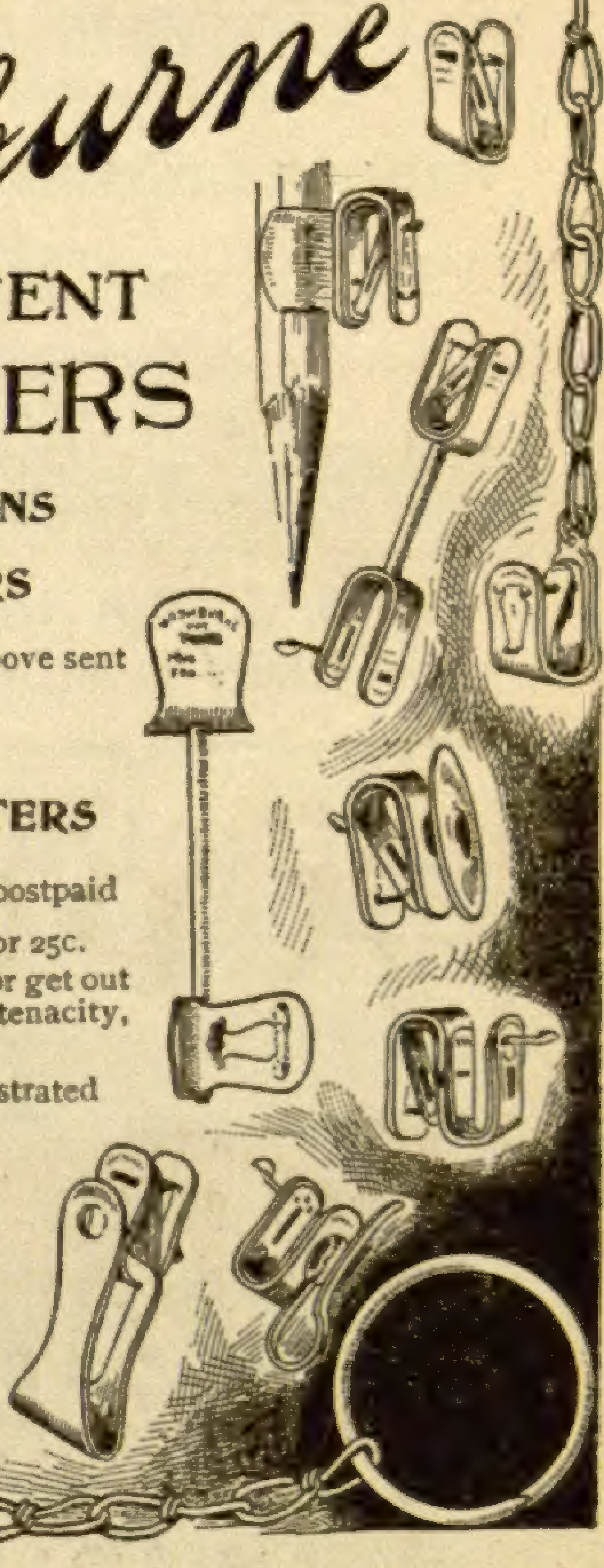

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